FINAL DEGREE PROJECT

Potential Impact of the FilmArray Multiplex PCR as compared to standard of care testing on Antimicrobial Therapy Guidance and Clinical Outcomes for Ventilator-Associated Pneumonia in Critically Ill Patients

A Two-Phased, Single Centre study

Cross-sectional Retrospective Observational Study using collected data from the ICU and Prospective, Open-labelled, Randomised Controlled Clinical Trial

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Contents

Abstract

Background Ventilator-associated pneumonia is common in critical care and can be life-threatening. Standard methods currently take at least 24 to 48 hours to identify the infectious etiology. The potential severity of this infection combined with a delay in pathogen identification results in administration of empirical broad-spectrum antibiotic agents, thus increasing the risk of multidrug-resistance acquisition and with limited assurance that the causative microorganism is being covered.

The FilmArray rapid multiplex PCR offers an alternative to address this issue by obtaining early pathogen identification as well as associated multiresistances, which may result in early narrow-spectrum antimicrobial stewardship (therefore decreasing the development of multidrug-resistant organisms) and clinical improvement of patients.

Objectives To determine if there is concordance between molecular testing with FilmArray and standard of care testing with conventional culture in pathogen detection and to determine if the use of FilmArray in ventilator-associated pneumonia allows an early start of appropriate targeted antibiotic treatment, improving the clinical outcome of patients.

Study participants The participants included in the study will be critically ill adults (≥18 years) diagnosed with ventilator-associated pneumonia being treated in the ICU of Santa Caterina Hospital and able to provide sufficient volume of airway specimen obtained for routine cultures and FilmArray.

Design and methods This is a two-phased, single-centre study that will be conducted in the ICU of Santa Caterina Hospital. The first phase will be a cross-sectional retrospective observational study, that will consist in reviewing the results of respiratory samples from patients diagnosed with VAP, which have been analysed in parallel with conventional culture and FilmArray, to determine the concordance between both techniques.

The second phase will be a prospective, open-labelled, randomised controlled clinical trial, that will consist in enrolling 200 patients through consecutive sampling, and randomly assigning them in a ratio 1:1 to a control group, who will be diagnosed with conventional culture and treated according to its results, and to an intervention group, who will be diagnosed with FilmArray plus conventional culture and will be treated according to FilmArray results. The time of intervention will be of 4 years. Independent outcome assessors will be in charge of drawing the conclusions of the study.

Keywords Ventilator-associated pneumonia, low respiratory tract infections, critical care, multidrug-resistant organisms, molecular testing, FilmArray, multiplex PCR.

Abbreviations

- ICU: Intensive care unit
- HAP: Hospital-acquired pneumonia
- VAP: Ventilator-associated pneumonia
- VAC: Ventilator-associated complications
- MDRO: Multidrug-resistant organisms
- XDR: Extremely drug-resistant
- MV: Mechanical ventilation
- ETT: Endotracheal tube
- ESBL: Extended-spectrum β-lactamases
- MSSA: Methicillin-sensible *Staphylococcus aureus*
- MRSA: Methicillin-resistant *Staphylococcus aureus*
- LRTI: Lower respiratory tract infection
- PCR: Polymerase chain reaction
- ARDS: Acute respiratory distress syndrome
- TBI: Traumatic brain injury
- COPD: Chronic obstructive pulmonary disease
- CNS: Central-nervous system
- GNB: Gram-negative bacteria
- CFU: Colony forming unit
- ITT: Intention-to-treat
- SOFA: Sequential organ failure assessment
- APACHE: Acute physiology and chronic health evaluation

1 | Introduction

1.1. Overview of Ventilation-associated Pneumonia

1.1.1. Definitions

Pneumonia

Pneumonia is an acute inflammation of the pulmonary parenchyma caused by an infectious agent, with alveolar occupation by inflammatory exudate. It is clinically defined as the presence of new lung infiltrate plus clinical evidence that the infiltrate is of an infectious origin, which include the new onset of fever, purulent sputum, leucocytosis and decline in oxygenation (1).

Pneumonia can be classified according the affected population in:

- Community-acquired pneumonia (CAP) → affects general population or patients who are admitted in a hospital for <48 hours.
- Hospital-acquired pneumonia (HAP), also referred to as "nosocomial" → affects hospital population and is developed ≥48 hours after hospital admission.

Hospital-acquired pneumonia (HAP)

Hospital-acquired associated pneumonia (HAP) is a pulmonary infection not incubating at the time of hospital admission and occurring ≥48 hours after admission (1,2). The concept of health-care associated pneumonia (HCAP), which included population who was hospitalized, residing in a nursing home or long-term care facility or in regular contact with healthcare facilities; has been removed from the most recent guidelines on management of VAP (1), reason why it will not be used in this study.

Ventilator-associated pneumonia (VAP)

Ventilator-associated pneumonia (VAP) is an infection of the lower respiratory tract that arises ≥48 hours after patients have been subjected to an invasive respiratory device (endotracheal intubation). It is considered of early-onset if it occurs in the first 4 days after endotracheal intubation and of late-onset if it occurs beyond that time (5^{th}-7^{th} day after endotracheal intubation) (3).

A respiratory infection related to mechanical ventilation must be suspected when respiratory symptoms appear (respiratory aggravation or purulent secretions) associated with clinical (fever, leukopenia, leucocytosis), biochemical (C-reactive protein or procalcitonin), radiological (increased lung density) or microbiological signs (positive respiratory culture) suggestive of a possible infectious origin.

Ventilator-associated complications (VAC)

Ventilator-associated complications (VAC) are defined as sustained increases in ventilator support (increase in fraction of inspired oxygen (FiO_2) by ≥20% or positive end-expiratory pressure (PEEP) by ≥2.5-3 cm/H_2O lasting ≥2 days) after ≥2 days of stable or decreasing settings (4,5).

This term has been developed to create a simple, objective measure of respiratory deterioration and its clinical importance lies in the fact that the appearance of ventilator-associated complications should lead to suspicion of VAP-onset or, in its absence, other serious complications such as acute respiratory distress syndrome, pneumothorax, pulmonary embolism, lobar atelectasis and pulmonary edema (6).

1.1.2. Epidemiology

Ventilation-associated pneumonia (VAP) is one of the main causes of nosocomial infection in the intensive care unit (ICU), making it one of the leading causes of antibiotic use in these units.

The epidemiological characterisation of VAP is difficult due to its complexity, the lack of surveillance systems in low/middle-income countries and the lack of uniformity of diagnostic criteria. It is reported to develop in 10% patients receiving invasive mechanical ventilation (7), with wide variations between different countries and ICUs (5-40%).

American hospitals, for instance, have reported to have 1-2,5 cases of VAP per 1000 ventilator days, whereas European centres report much higher rates (18,3 VAP episodes per 1000 ventilator days) (8).

According to the Envin-ICU records (9), the Intensive Care Unit of Santa Caterina Hospital (IAS) has an incidence density of 8,77 cases of VAP per 1000 ventilator days, of which 25% are due to MDRO.

To emphasize this, it has to be noted that, in an international basis, resistance rates are alarmingly increasing, making it more difficult to match the administered antibiotic with the susceptibility of the present pathogen and, therefore, increasing mortality associated with treatment inadequacy (10).

Although all-cause mortality associated with VAP has been reported to be as high as 50%, the mortality attributable to VAP is mainly driven by the patient's underlying conditions and illness severity and has been estimated at 13%.

Further to this, patients with VAP face prolonged duration of mechanical ventilation and ICU stay, longer hospital course and incur higher healthcare costs than similarly ill patients without VAP (11).

1.1.3. Etiology

The etiology of VAP varies depending on several factors, such as length of hospital stay, previous antimicrobial treatment, duration of mechanical ventilation, local epidemiology and potential outbreaks, among others (8,12).

HAP/VAP can be caused by a wide spectrum of bacterial pathogens, can be polymicrobial and are rarely due to viral or fungal pathogens in immunocompetent hosts (2).

Bacterial infections

Etiological agents

Overall, 80% of the cases are caused by *S. aureus, Pseudomonas aeruginosa, Acinetobacter, Klebsiella* and *Enterobacter* spp.. The most common etiological agents for both HAP and VAP are (12):

- Gram-negative bacteria: *Pseudomonas aeruginosa, Acinetobacter spp., Klebsiella* spp., *Enterobacter* spp., *Haemophilus* spp., *Escherichia coli* and, to a lesser extent, *Serratia marcescens,* and *Stenotrophomonas maltophilia*.
- Gram-positive bacteria: methicillin-sensitive *Staphylococcus aureus (MSSA)* and methicillin-resistant *Staphylococcus aureus (MRSA), Streptococcus pneumoniae* and *Enterococcus* spp.

Time of onset of pneumonia

The time of onset of pneumonia is considered one of the factors that determines the composition of the pathogens involved in VAP, with implications in the patient's clinical outcomes (2,3,8,12):

- Early-onset (<5 days): low risk of MDRO (indigenous oropharyngeal flora of the patient) → *Streptococcus pneumoniae, Haemophilus influenzae, Staphylococcus aureus, BGN* (*Escherichia coli, Klebsiella pneumoniae*). It usually carries a better prognosis and is more likely to be caused by antibiotic-sensitive bacteria.
- Late-onset (≥5 days): high risk of MDRO (hospital infectious microorganisms) → *Pseudomonas aeruginosa, MRSA, ESBL-producing Klebsiella pneumoniae, Legionella pneumophila, Acinetobacter* spp. It is more likely to be caused by multidrug-resistant pathogens, and is associated with increased patient mortality and morbidity.

This theory has been subjected to validation in several recent studies that showed that there were no differences in the isolated microorganisms from early and late samples (13,14). These studies,

however, varied in their definitions of important concepts such as risk factors for MDRO, which should take precedence over the distinction between early and late-onset pneumonia, and the definition of "time zero", which should be based on hospital admission as starting point, rather than intubation, as this may have resulted in a patient already colonized with typically nosocomial pathogens.

Nonetheless, the new ISDL/ATS guidelines have determined that the reviewed evidence suggests that overall, patients who develop VAP after >5 days of hospitalization are at higher risk of infection with MDRO than patients who develop VAP earlier in their hospitalization.

The type of ICU population (medical, surgical and trauma) is associated with specific causal agents (**TABLE 1**) and the use of mechanical ventilation establishes the differences in prevalence of the main etiological agents of each entity (HAP or VAP) (12,13).

Table 1. Specific pathogens according to the type of ICU population

ICU population	Associated specific pathogen
Non-intubated patients (HAP)	*S. aureus, Klebsiella* spp.
Mechanically ventilated patients (VAP)	*Pseudomonas aeruginosa, Acinetobacter* spp.
Neurosurgery and traumatic brain injury (TBI)	*A. baumanii, S. aureus*
Trauma	*Haemophilus* spp., *S. pneumoniae*
Chronic obstructive pulmonary disease (COPD)	*H. influenzae, Moraxella catarrhalis*
Bronchiectasis	*P. aeruginosa, S. aureus*

In conclusion, knowledge of the microbial epidemiology of each intensive care unit is crucial in order to administer the appropriate antibiotics to treat these infections (3).

Fungal and viral infections

In the remaining 20% of cases, VAP can be caused by fungal and viral pathogens. The role they play in the etiology of VAP is not entirely clear.

Fungal infections

Hospital-associated pneumonia due to fungi, such as *Candida* species and *Aspergillus fumigatus*, is uncommon in immunocompetent patients, but may occur in organ transplant or immunocompromised, neutropenic patients (2).

Isolation of *Candida* spp. in respiratory samples is common in intubated patients, but usually represents colonization of the lower respiratory tract, rather than the cause of VAP and rarely requires antifungal therapy (12). Despite this, it is considered that colonization by *Candida* favours the development of VAP by *P. aeruginosa* and other MDRO and implies bad prognosis (8,12).

Aspergillus species infections suggest possible airborne transmission by spores, and may be associated with an environmental source such as contaminated air ducts or hospital construction (2).

Viral infections

The incidence of HAP and VAP due to viruses is also low in immunocompetent hosts. Outbreaks of HAP and VAP due to influenza, parainfluenza, adenovirus and respiratory syncytial virus have been reported and are usually seasonal.

Influenza A is probably the most common viral cause of HAP and VAP, and the resulting pneumonia may be due to the virus, to secondary bacterial infection, or both. Respiratory syncytial virus outbreaks of bronchiolitis and pneumonia are more common in children's wards (2)

Herpes simplex virus (HSV) and *Cytomegalovirus* (CMV) are frequently detected in immunocompetent and immunocompromised patients admitted to the ICU and can cause pneumonia in patients with prolonged intubation. They should be suspected in patients with ARDS and those treated with glucocorticoids, especially if no bacterial agent has been isolated (8,12).

In the past 2 years, *SARS-CoV-2* has been a novel responsible cause of acute respiratory distress syndrome, leading patients to ICU admission requiring invasive ventilation and thus, increasing the risk of VAP. The incidence of VAP due to SARS-CoV-2 infection is higher (33%) in comparison with general ICU population (10%), but similar to that of ICU ARDS patients in pre-COVID-19 period (15), reason why this etiological agent should be considered in when suspecting VAP of viral etiology.

1.1.4. Emergence of multidrug-resistant organisms (MDRO)

Rates of VAP due to multidrug-resistant pathogens have increased dramatically in the last years, especially in hospitalized transplant patients and in intensive care units (2), increasing mortality associated to treatment inadequacy.

The resultant fear of treatment failure due to antimicrobial resistance leads to the empirical prescription of broad-spectrum antibiotics to increase the possibility of matching the administered antibiotic with the susceptibility of the present pathogen. This fact favours, in a counterproductive way, the

development of more multidrug-resistant organisms whereas, in reality, it would be much better to provide early administration of antibiotics with the narrowest possible spectrum (16).

Multidrug-resistance (MDR) is defined as *in vitro* resistance against 1 or more agents in 3 or more antibiotic classes. Some specific factors have been associated with an increased risk of multidrug-resistant VAP, which are displayed in **Table 2** and include prior antibiotic use within 90 days, septic shock at time of VAP onset, ARDS and acute renal replacement therapy preceding VAP, among others.

Table 2. Risk factors for multi-drug resistant organisms (MDRO). Adapted from (2,12)

Risk factors for multi-drug resistant organisms (MDRO)
· Antimicrobial therapy in preceding 90 days · Prolonged hospitalization → duration of current hospitalization episode ≥5 days · High prevalence of antimicrobial resistance in the community or in the specific hospital unit[1] · Immunosuppressive disease or therapy · Previous colonization by multiresistant pathogen · Presence of septic shock at time of VAP onset · ARDS (acute respiratory distress syndrome) prior to the development of VAP · Acute renal replacement therapy before the onset of VAP · Specific risk factors – *Pseudomonas aeruginosa*: structural defects in the lung, malnutrition, recent antimicrobial treatment, glucocorticoid therapy, prolonged ICU stay – *Staphylococcus aureus*: traumatic brain injury, coma, diabetes mellitus, flu, kidney failure, intravenous drug use (IVDU) – *Streptococcus pneumoniae*: age <2 or >65 years, alcoholism, immunosuppression or major associated comorbidities, contact with children in nurseries, recent treatment with β-lactams.

[1] High prevalence of antimicrobial resistance has been defined in thresholds to balance the need for effective initial antibiotic therapy against the risks of excessive antibiotic use. Every unit can elect to adjust the threshold in accordance with local values and preferences. High prevalence of antimicrobial resistance is considered in:

- Units where local resistance is unknown (due to unavailability of local antimicrobial susceptibility rates) as, if the local resistance is unknown, it must be considered high until proven otherwise.
- Units where >10-20% of S. aureus isolates are methicillin resistant.
- Units where >10% of gram-negative isolates are resistant.

The reason for the lower threshold for gram-negatives compared with-gram positives is because gram negatives are more frequently implicated in VAP, hence, there is increased risk for inadequate empiric gram-negative coverage.

Mechanisms of antibiotic resistance

Improved infection control has reduced the National Health Service (NHS)'s burden of methicillin-resistant *Staphylococcus aureus* (MRSA) and *Clostridium difficile*, but resistance rates among Gram-negative bacteria are rising alarmingly on an international basis (10).

Methicillin-resistant *Staphylococcus aureus* (MRSA) produces a penicillin-binding protein with reduced affinity for β-lactam antibiotics that is encoded by the mecA gene. Strains with mecA are resistant to all commercially available β-lactams and many other anti-staphylococcal drugs.

Pseudomonas aeruginosa is the most common MRD gram-negative bacterial pathogen causing VAP. It has intrinsic resistance to many antimicrobial agents mediated by multiple efflux pumps, decreased expression of an outer membrane porin channel (OprD) or, in some strains, IMP-type enzymes, which confer the acquisition β-lactamases active against carbapenems, antipseudomonal penicillins and cephalosporins, raising a big concern in this regard. At present, some MDR isolates of P. aeruginosa are susceptible only to polymyxin B.

Klebsiella is intrinsically resistant to ampicillin and other amino-penicillins and can acquire resistance to cephalosporins and aztreonam by the production of extended-spectrum β-lactamases (ESBLs) or, in 5-10% of strains, with AmpC-type enzymes. These strains are usually susceptible to carbapenems but may become resistant through loss of an outer membrane porin.

K. pneumoniae, with KPC carbapenemases, and *Enterobacter* species, with NDM carbapenemases, are explosively increasing, most of them being susceptible in vitro only to a few antibiotics, such as colistin and tigecycline, which have significant toxicity and efficacy limitations.

Acinetobacter species are generally less virulent than P. aeruginosa, but their increasing resistance to commonly used antimicrobial agents has become a general concern. More than 85% of isolates are susceptible to carbapenems, but their resistance is increasing due either to IMP-type metalloenzymes or carbapenemases of the OXA type.

The available data on the mechanisms of antibiotic resistance of these bacterial pathogens (2,10) provide new insight into their adaptability.

1.1.5. Pathogenesis

Origin of the causative agents

The causes of VAP are multifactorial (3,13). The origin of the causative agents and infections can be:

- Endogenous: primary (usual bacterial microbiota of the patient) or secondary (replacement of the usual microbiota by hospital microorganisms)
- Exogenous: inhalation of infected aerosols, contaminated nebulizers, ventilator tubes, anaesthesia equipment, bronchoscopes, hands or clothing of healthcare personnel (12)

Production mechanisms

1. Aspiration

Most cases of HAP are caused by the micro-aspiration of oropharynx colonisation agents and, occasionally, the upper gastrointestinal tract. Hematogenous dissemination from infected vascular catheters and bacterial translocation from the gastrointestinal lumen are much less common mechanisms of infection.

In **hospitalized patients**, there is a combination of depressed immune function, suppression of swallowing and cough reflex and impaired clearance of the mucociliary system, that together with the presence of comorbidities, malnutrition and pathogenic microorganisms, contribute significantly to aspiration and subsequent development of pneumonia (2).

In **intubated patients**, the placement of the endotracheal tube keeps the vocal cords open and allows the passage of subglottic secretions. In these patients, the micro-aspiration of oropharyngeal secretions occurs around the tracheal cuff. The presence of a tracheal cuff prevents normal trachea clearance and the passage of mucus to lower respiratory tract. As a result of the cuff's inflation, oropharyngeal secretions and gastric content caused by reflux will accumulate on its surface.

Although there is an estimated safe pressure that prevents the passage of these fluids, small folds are often formed in the cuff, allowing leakage of secretions into the lower airways (3,13,17).

2. Direct inoculation

Direct inoculation can occur during the intubation procedure through the endotracheal tube (ETT), suction probes or the fiberoptic bronchoscope, as they are invasive devices that can carry bacteria from the external environment to the lower respiratory tract through direct contact (17).

3. Biofilm formation

During the time of intubation, bacterial pathogens can grow on the inner surface of the endotracheal tube and aggregate to form a biofilm that covers the inside of the tube. The biofilm protects pathogens from the action of antibiotics and from the patient's immune defense mechanisms and acts as a reservoir of bacteria that can be easily detached by the effect of mechanical ventilation and suction probes and, through gravity, can potentially migrate to the lungs (2,3,17).

4. Contaminated respiratory equipment and contaminating secretions

In addition to this, the formation of droplets resulting from water condensation occurs inside the ETT during ventilation, and the ventilation circuit can descend into the lungs, carrying bacteria resulting from the formation of biofilm in the ETT or circuit contamination due to poor cleaning of the equipment or inadequate hygiene (3,17).

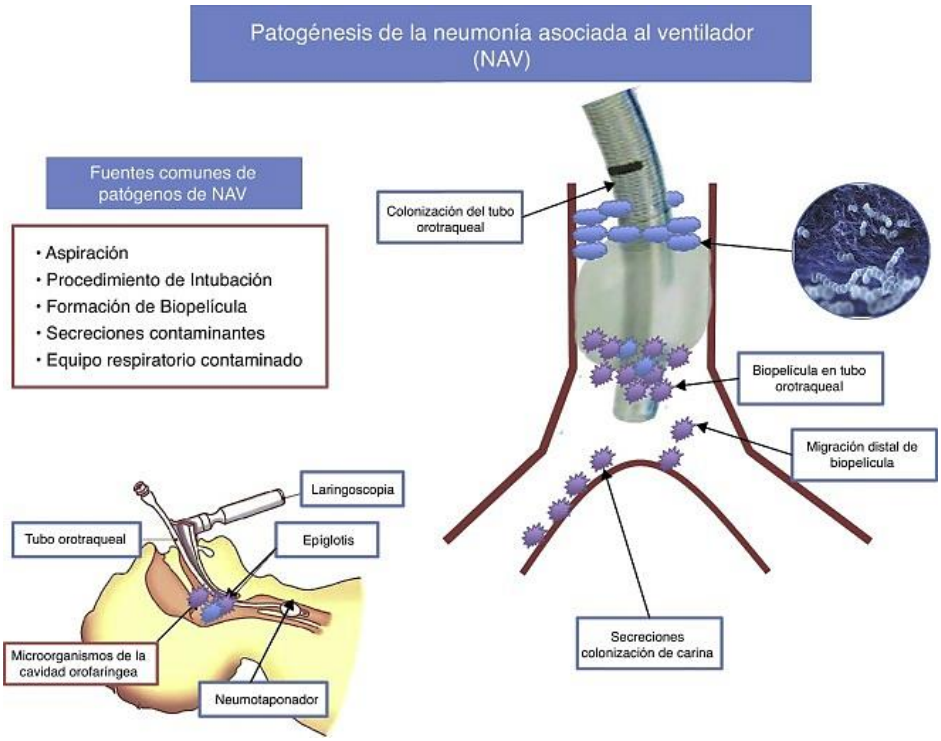


Figure 1. Pathogenesis of ventilator-associated pneumonia. Image taken from article (6).

1.1.6. Risk factors for development of VAP

In hospitalized patients, especially in those undergoing mechanical ventilation, there are different risk factors that must be taken into account for patient management (2,12).

1. **Intrinsic risk factors** (patient related) → situations that alter patient's defense mechanisms
   - Advanced age, malnutrition, alcoholism and smoking
   - Underlying chronic diseases: COPD and other pre-existing pulmonary disease, central nervous system and neuromuscular diseases, diabetes mellitus, chronic renal failure or dialysis.
   - Traumatic brain injury, impaired level of consciousness and coma
   - Hypotension and shock, acidosis, ARDS
   - Multiple organ system failure and immunosuppression
2. **Extrinsic risk factors** (treatment related) → diagnostic and therapeutic manipulations
   - Artificial airways: endotracheal tubes, reintubation, tracheostomy and mechanical ventilation
   - Enteral feeding and nasogastric tubes
   - Aspiration and supine body position
   - Prolonged hospital stay
   - Aerosol sprays
   - Prolonged or inappropriate antibiotic therapy
   - Treatment with gastric antisecretory agents (increase in pH and gastrointestinal colonization), cytotoxics, corticosteroids, CNS sedatives, etomidate in intubation (18), transfusion of >4 units of hemoderivatives
   - Major or complicated surgery
   - Poor in-hospital control of infections: lack of handwashing, no replacement of gloves, poor isolation of the patient

1.1.7. Diagnosis

VAP diagnosis is traditionally defined by the concomitant presence of three criteria:

- Clinical suspicion (fever, respiratory decline, productive cough)
- New or progressive and persistent radiographic infiltrates
- Positive microbiological cultures from lower respiratory tract specimens (8)

Clinical diagnosis

Clinical manifestations

Clinical assessment is the first step to diagnose VAP, which should be considered whenever there are new signs of respiratory deterioration potentially attributable to infection. All patients should have a comprehensive medical history obtained and undergo physical examination to determine severity, exclude other potential sources of infection and to reveal the presence of specific conditions that can influence the likely etiologic pathogens (2).

Clinical findings suggesting infection include: new onset of fever (>38,2ºC), purulent sputum, leucocytosis (>12.000/mm^3) or leukopenia (<4.000/mm^3), presence of immature forms (>10%), focal abnormal lung auscultation, decline in oxygenation (PO_2/FiO_2 <250 in an acute patient, or increase of >10% FiO_2 compared to the previous), increase in minute ventilation and hemodynamic instability (increased need for vasopressors to maintain blood pressure).

Many criteria for suspecting VAP exist, but their usefulness, alone or in combination, is not sufficient to diagnose it, especially in ventilated patients where symptoms may be less evident due to the increased susceptibility of ICU patients (in immunocompromised, malnourished, hemodynamically unstable or glucocorticoid-treated patients, temperature may be normal or even decreased) (19).

Scores have been proposed to improve diagnostic accuracy, the most used being the Clinical Pulmonary Infection Score (CPIS), an index in which seven different clinical variables (temperature, white blood cell count, appearance of respiratory secretions, oxygenation, radiographic infiltrates, Gram stain and semiquantitative cultures of tracheal aspirates) are assessed on the first day that the diagnostic possibility arises and again 72 hours later (**TABLE 3**)

The total sum of points can vary between 0 and 12, with a score higher than 6 being significantly associated with the existence of VAP (2,8). Recent guidelines, however, do not recommend CPIS to diagnose VAP, given its variability, its moderate sensitivity and specificity and its lack of validation for entities such as ARDS or trauma patients (2,20).

Table 3. Clinical Pulmonary Infection Score (CPIS) for VAP

Item	Value	Score
Temperature (ºC)	36,5-38,4	0
	38,5-38,9	1
	≥39 or ≤36	2
White blood cell count	4-11	0
	<4 or >11	1
	Either <4 or >11 plus band forms ≥500	2
Tracheal secretions	<14+	0
	≥14+	1
	≥14+ plus purulent secretions	2
Oxygenation PaO_2/FiO_2 (mmHg)	>240 or ARDS	0
	≤240 and no ARDS	2
Pulmonary radiography	No infiltrate	0
	Diffuse or patchy infiltrate	1
	Localized infiltrate	2
Culture of tracheal aspirate specimen. Semi-quantitative, 0, 1, 2, or 3+	Pathogenic bacteria cultured ≤1 or no growth	0
	Pathogenic bacteria cultured >1+	1
	Pathogenic bacteria cultured >1+ plus same pathogenic bacteria on gram stain >1+	2
	Total	/12

Radiographic chest imaging

Almost all definitions for suspecting VAP include radiographic criteria, as new or progressive and persistent infiltrates, which usually present as bilateral bronchopneumonic focus. Its absence significantly lowers the probability of VAP and can guide the clinician to alternative causes of inpatient respiratory decline.

Chest X-ray, however, has complex interpretation and low sensitivity and specificity for VAP, especially in intubated patients in whom the image is taken with portable equipment, reason why VAP should be considered whenever there are new signs of respiratory deterioration potentially attributable to infection with or without new/progressive infiltrates (8,11,12).

Computed tomography (CT) is more sensitive, but a strategy based on systematic lung CT-scan has obvious drawbacks, the main issues being feasibility, maintaining patient safety during transport and availability. Lung ultrasound has recently been proposed as a diagnostic aid for VAP, but data on its sensitivity and specificity are lacking.

Laboratory testing

Laboratory tests usually reveal left-deviating leucocytosis or leukopenia and, as in any serious septic process, increased liver enzymes and coagulation disorders can also be observed. In mechanically ventilated patients, sustained hypoxemia is starting to be considered a *sine qua non* condition for the diagnosis of VAP, in which the decrease in PaO_2 or PaO_2/FiO_2 ratio frequently precedes radiological infiltrates.

Biomarkers such as C-reactive protein, procalcitonin, soluble triggering receptor expressed on myeloid cells (sTREM-1), IL-1β and 8, G-CSF and plasminogen activator inhibitor 1 (PAI-1) have been proposed as diagnostic markers for VAP.

Nonetheless, they lack accuracy and their use is, to date, restricted by the most recent guidelines to help guide the clinical course of patients, which, in no case, should be used to diagnose the patient or make therapeutic decisions regarding antimicrobial stewardship (1,8,21).

Microbiological diagnosis

Given that clinical criteria alone have a moderate predictive value, once VAP is suspected they should be complemented with the performance of microbiological sampling, which is the second step of the diagnostic workup.

In hospital-acquired pneumonia, as opposed to community-acquired pneumonia, it is of utmost importance to achieve an etiological diagnosis (in 10-20% of VAP no bacterial isolation is achieved).

Definitive microbiological diagnosis is based on the isolation of a potentially causative microorganism for pneumonia in blood, pleural fluid or a valid sample of respiratory secretions. All guidelines recommend obtaining respiratory samples before starting antibiotic treatment, since it is known that if samples are obtained afterwards, the results may be altered or emerge negative (1,2,20). Regardless of this, in no case should this sample collection result in a delay in the initiation of empirical treatment.

In all patients, the following non-invasive tests should be performed (22):

- *Blood cultures* (x2): blood cultures are positive in about 20% of cases, but in critically ill patients with venous and urinary catheterizations, the isolated microorganism is not always necessarily responsible for the pulmonary infection. For example, Candida and Enterococcus species are not known to cause pneumonia, so detecting these pathogens in bloodstream may direct the clinician to a separate and previously unsuspected site of infection (catheter-related bloodstream infection)
- *Sample of respiratory secretions* → its performance is always mandatory

In case of pleural effusion, a *study of pleural fluid* could be of used, especially considering that, in contrast with blood cultures, isolation of a microorganism in pleural fluid culture usually confirms the etiological diagnosis of pneumonia.

If suspected *S. pneumoniae* and *L. pneumophila urinary antigen tests* can be performed to confirm its presence. In patients on corticosteroid therapy, *Aspergilllus* should be searched for in sputum or galactomanate should be determined in serum. To complete the bacteriological study, it would be advisable to request a serology for L. pneumophila, S. pneumoniae and respiratory viruses.

Lower respiratory tract sampling

Description of microbiological results

The microbiological results of the obtained samples from the lower respiratory tract can be expressed different ways depending on how they have been analysed:

- **Qualitative results**: define the presence (+) or absence (–) of pathogenic organisms in the culture. The advantage is that they are easy to perform. The drawback is that they have little positive predictive value (PPV), they do not allow knowing the degree of colonization and may overestimate the presence of bacteria so, as a result, potentially lead to unnecessary use of antibiotics and promote antibiotic resistance than diagnostic techniques based on quantitative results (2,23), reason why their use is discouraged.
- **Semiquantitative results**: describe bacterial growth as light, moderate or heavy, and they are preferred over quantitative samples to reduce cost and patient harm associated with quantitative and invasive testing (11). According to American guidelines (1), **non-invasive sampling with semiquantitative cultures is the preferred methodology** to diagnose VAP and base the antimicrobial treatment.
- **Quantitative results**: define a threshold concentration of bacterial growth to distinguish between bacterial colonization and active respiratory infection, below which it is assumed to be due to colonization or contamination, and above which VAP is diagnosed and the microorganism is recognised as causative.

  The advantage is that they have better diagnostic utility to determine the presence of pneumonia and allow knowing the degree of microbial load of the sample and potentially leading to less antibiotic exposure if growth below defined thresholds is used as a trigger to stop antibiotics.

  Nevertheless, they have the disadvantage that a false negative can trigger the cessation of appropriate therapy and lead to a failure to treat. For this reason, all quantitative sampling methods should be obtained before any antibiotic therapy is started.

Available sampling methods

A major problem with all studies for VAP diagnosis is the absence of a Gold Standard with which diagnostic results can be compared and even the best criteria for the presence of pneumonia and immediate postmortem histologic evaluation with microbiologic confirmation, can be inaccurate (2). The sampling methods that are currently available are:

1. Non-invasive techniques → proximal sampling methods
   a. Sputum samples (not available)
   b. Endotracheal aspirate: semiquantitative sputum
2. Invasive sampling methods → distal sampling methods
   a. Bronchial aspirate (BAS)
   b. Protected Specimen Bronchial Brushing (PSB)
   c. Bronchoalveolar lavage (BAL)

Table 4. Sampling techniques for microbiological diagnosis of VAP

Technique	Definition	Positivity
Non-invasive techniques (proximal sampling)	**Endotracheal aspirate**: aspiration of respiratory secretions through the endotracheal tube (easy to perform)	$\geq 10^6$ CFU/ml
Invasive techniques (distal sampling)	**Bronchial aspirate (BAS)**: invasive equivalent of tracheal aspirate	$\geq 10^6$ CFU/ml
	Protected specimen bronchoalveolar brush (PSB): brush protected by a polyethylene glycol cap through which the sample is obtained, avoiding contamination from the upper airway or the fibreoptic bronchoscope (currently in disuse)	$\geq 10^3$ CFU/ml
	Bronchoalveolar lavage (BAL): study of the resulting fluid from instilling saline into the lungs through a bronchoscope (3 syringes of 50cc → total of 150cc). It is considered that the first aliquot represents bronchial cellularity and the following ones, alveolar cellularity. The last sample is representative of the parenchymal infectious process, and is the one that should be sent for culture, identifying the lobe from with it is extracted.	$\geq 10^4$ CFU/ml
	Lung biopsy: procedure in which a sample of lung tissue is removed for later analysis, using an open (surgical) or closed (needle) method. Only used in the event of poor evolution. Transbronchial biopsies are of worse quality since they drag mucosal debris.	Qualitative result

Non-invasive techniques

Direct examination and Gram stain: a high-quality Gram stain from a respiratory specimen with numerous organisms provides further support for the diagnosis of VAP (1), but its sensitivity and specificity is limited and the absence of microorganisms does not reliably exclude VAP, reason why it is important to also review culture result (8).

Culture of respiratory secretions: Culture of sputum or tracheal aspirates must be interpreted with caution, since these samples are often contaminated by microorganisms that colonize the oropharyngeal cavity or central airways (trachea and large bronchi).

- *Sputum cultures* are usually obtained in non-intubated patients who are capable of producing a sufficient sample and, to be valuable, they must be of good or optimal quality, which means that they have to be representative of the lower airways → >25 polimorphonuclears and <10 epithelial cells/low magnification field (Murray-Washington's criteria, available in **Annex 3**). In intubated patients, the obtention of an adequate sputum sample is not available and therefore other methods, such as endotracheal aspirate, have to be used to perform non-invasive sampling.
- *Endotracheal aspirate:* quantitative analysis of tracheal aspirates allows to distinguish between bacterial colonization and active respiratory infection (concentrations equal to or greater than 10^6 UFC/ml), with comparable results to those obtained with invasive procedures, such as BAL/PSB.

Invasive techniques

The invasive techniques that must be considered are: bronchial aspirate (BAS), Protected Specimen Bronchial Brushing (PSB), Bronchoalveolar lavage (BAL) and lung biopsy (transthoracic pulmonary puncture or open lung biopsy are rarely necessary).

Bronchoalveolar lavage (BAL) or *protected specimen bronchial brush (PSB)* are the indicated techniques in intubated or tracheostomized patients who do not respond to initial empiric antibiotic treatment and can be performed with the bronchoscope or blindly, without significantly altering the diagnostic accuracy. It is accepted that a pathogenic agent has a causal or infective role when it is found in BAL fluid in concentrations equal to or greater than 10^4 UFC/ml.

In addition to bacterial cultures, the BAL allows the study of opportunistic microorganisms and viruses, which is especially useful in immunocompromised patients, with high sensitivity and specificity (90%). The presence of intracellular microorganisms in more than 2% of the macrophages or neutrophils collected in the BAL is highly specific for HAP.

Although its use is currently decreasing, the *protected specimen bronchial brush (PSB)* allows the obtention of samples from the lower airways that are not contaminated by oropharyngeal flora, with similar sensitivity and specificity to that of BAL. The limit to distinguish between colonization and infection has been established at 10^3 CFU/ml when using this type of catheter.

The sensitivity of these procedures markedly decrease when used in patients who have received or are receiving antimicrobials.

The most recent recommendation from American guidelines (1) suggests non-invasive sampling (endotracheal aspiration) with semiquantitative cultures to diagnose VAP rather than invasive techniques and quantitative cultures.

This suggestion is based on the fact that, although invasive sampling has proven to lead to more antibiotic changes and more antibiotic-free days, outcomes are similar regardless of whether specimens are obtained invasively or non-invasively and whether cultures are performed quantitatively or semi-quantitatively.

Advantages and disadvantages of the sampling methods

Distal sampling methods with invasive techniques have several disadvantages such as the need for qualified personnel to perform these procedures, even though it is now a conditional skill to become an intensivist in many countries, the potential risks for the patient (hypoxemia, barotrauma, bleeding) and the associated costs, especially when using disposable bronchoscopes.

However, they also have remarkable advantages, as the use of bronchoscopic BAL combined with quantitative cultures may achieve more reliable identification of causative agents, with a higher specificity than qualitative sampling methods and allows sufficient fluid return to perform complementary analyses (cytology, albumin levels, viruses identification, galactomanan determination, procollagen III in ARDS patients).

Proximal sampling methods with non-invasive techniques also present some disadvantages. On one hand, they may overestimate the presence of bacteria, potentially lead to unnecessary use of antibiotics and promote antibiotic resistance. On the other hand, the advantages are that they can be performed more quickly and simply compared to bronchoscopy, with fewer complications and resources.

Table 5. Advantages and disadvantages of EAS and BAL. Adapted from Article (8)

	Endotracheal aspirate with semiquantitative or quantitative culture	**Bronchoalveolar lavage** with quantitative culture
Advantages	Cheap Tolerance Easy to perform Sensitive	Specific Less antibiotics Large sample
Disadvantages	Specificity Antibiotic use Only microbiological exams	Sensitivity Cost Tolerance Special skills

1.1.8. Therapeutic management

The treatment of VAP should be a two-step process (8):

- First step → empiric treatment, chosen by disease severity and risk factors of MDRO (**TABLE 2**)
- Second step → definitive treatment, for which clinicians should try to avoid overuse of antibiotics

Empiric treatment

The empiric treatment should cover methicillin-susceptible *S. aureus* and gram-negative bacilli such as *Pseudomonas aeruginosa* (1,11). The choice and timing of antimicrobial agents used should take into account five parameters:

1. Severity of the current illness
2. Type and number of underlying diseases and their severity
3. Risk factors for MDRO
4. Local pattern of antimicrobial susceptibility
5. Previously used antibiotics

In patients with early-onset VAP (<5 days), no risk factors for MDRO and being treated in units with low-prevalence of MDRO it is recommended to start monotherapy with a non-antipseudomonal narrow-spectrum antibiotic:

- Non-antipseudomonal β-lactam with a β-lactamase inhibitor
- Third generation cephalosporin (ceftriaxone)
- Antipneumococcal fluoroquinolone (levofloxacine or moxifloxacine)
- Non-antipseudomonal carbapenem (ertapenem)

In patients with late-onset VAP (≥5 days) or risk factors for MDRO it is recommended to start combined treatment with broad-spectrum antibiotics with antipseudomonal activity:

a) Broad-spectrum β-lactam targeting *Pseudomonas* and ESBL-producing *Enterobacteriaceae*
   - 3rd and 4th generation cephalosporins (cefepime or ceftazidime)
   - Carbapenem (imipenem or meropenem)
   - Penicillin associated with a β-lactamase inhibitor (piperacillin-tazobactam)

b) Non β-lactam antipseudomonal agent
   - Fluoroquinolones (levofloxacin or ciprofloxacin)
   - Aminoglycosides (amikacin or tobramycin)
   - Polymyxins (colistin) → they should be reserved for settings where there is a high prevalence of multidrug resistance and local expertise in using this medication (1,8,12,17)

Monotherapy is suggested in patients with VAP due to *P. aeruginosa* who are not in septic shock or at high risk of death, without risk factors for MDRO and being treated in ICUs where <10% of gram-negative isolates are resistant to the agent being considered for monotherapy, except if patient has structural lung disease increasing the risk of gram-negative infection (bronchiectasis or cystic fibrosis).

If the patient remains in septic shock or at high risk of death when the results of antibiotic susceptibility testing are known, combination therapy using two antibiotics to which the isolate is susceptible is preferred (1).

It is recognised that carbapenems may not be the best empiric agent of choice for VAP, since they are associated with selection of *C. difficile* and a significant increase in antibiotic resistance. Therefore, their use should be limited only to patients with true infection of ESBL-producing *Enterobacteriaceae*.

Inhaled antibiotic therapy

Nebulisation of antibiotics has grown in recent years, but the ideal candidates to receive this treatment are not well defined. To date, nebulized antibiotics cannot be recommended as an alternative to the intravenous route, partly because data are lacking on this indication, because 10-20% of patients with VAP are bacteriemic and partly because multiple and repeated daily use of nebulisation may prolong duration of mechanical ventilation.

The use of nebulised antibiotics as an adjunctive treatment for VAP due to traditional pathogens is not recommended, and it should therefore be restricted to patients with VAP due to XDR-Gram-negative pathogens susceptible only to aminoglycosides or polymyxins. However, it is reasonable to consider adjunctive inhaled antibiotic therapy as a treatment of last resort for patients who are not responding to intravenous antibiotics alone, whether the infecting organism is or not multi-drug resistant (1,8).

Table 6. Suggested empiric treatment options for clinically suspected Ventilator-Associated Pneumonia in units where empiric Methicillin-Resistant *Staphylococcus aureus* coverage and double antipseudomonal/Gram-negative coverage are appropriate. Adapted from 2016 Clinical Practice Guidelines by IDS/ATS.

A. Gram-Positive Antibiotics With MRSA Activity	B. Gram-Negative Antibiotics With Antipseudomonal Activity: β-Lactam–Based Agents	C. Gram-Negative Antibiotics With Antipseudomonal Activity: Non-β-Lactam–Based Agents
Glycopeptides[a] Vancomycin 15 mg/kg IV q8–12h (consider a loading dose of 25–30 mg/kg × 1 for severe illness)	Antipseudomonal penicillins[b] Piperacillin-tazobactam 4.5 g IV q6h[b]	Fluoroquinolones Ciprofloxacin 400 mg IV q8h Levofloxacin 750 mg IV q24h
OR	OR	OR
Oxazolidinones Linezolid 600 mg IV q12h	Cephalosporins[b] Cefepime 2 g IV q8h Ceftazidime 2 g IV q8h	Aminoglycosides[a,c] Amikacin 15–20 mg/kg IV q24h Gentamicin 5–7 mg/kg IV q24h Tobramycin 5–7 mg/kg IV q24h
	OR	OR
	Carbapenems[b] Imipenem 500 mg IV q6h[d] Meropenem 1 g IV q8h	Polymyxins[a,e] Colistin 5 mg/kg IV × 1 (loading dose) followed by 2.5 mg × (1.5 × CrCl + 30) IV q12h (maintenance dose) [135] Polymyxin B 2.5–3.0 mg/kg/d divided in 2 daily IV doses
	OR	
	Monobactams[f] Aztreonam 2 g IV q8h	

Choose one gram-positive option from column A, one gram-negative option from column B, and one gram-negative option from column C. Note that the initial doses suggested in this table may need to be modified for patients with hepatic or renal dysfunction.

Abbreviations: CrCl, creatinine clearance; IV, intravenous; MRSA, methicillin-resistant *Staphylococcus aureus*.

Pathogen-specific therapy

Multidrug-resistance is defined as in vitro resistance against ≥1 agents in ≥3 antibiotic classes.

1. MRSA: vancomycin or linezolid → if there is suspicion, risk factors for antimicrobial resistance (**Table 2**), unknown or high in-hospital prevalence (patients being treated in units where >10-20% of S. aureus isolates are methicillin resistant) for MRSA, vancomycin or linezolid should be added to the above. The final choice for empiric MRSA coverage should rest upon blood cell counts, renal function, concurrent prescriptions for serotonin-reuptake inhibitors and cost.
2. Extended-spectrum β-lactamase (ESBL)-producing bacteria or other multiresistant gram negative bacteria → the choice of antibiotic for definitive therapy should be based upon the results of antimicrobial susceptibility testing and patient-specific factors. The most widely used antibiotics are antipseudomonal carbapenems (imipenem-cilastatin and meropenem).
3. *Pseudomonas aeruginosa:* the choice of antibiotic for definitive therapy should be based upon the results of antimicrobial susceptibility testing. There is controversy regarding the use of fluoroquinolones or aminoglycosides, but monotherapy with aminoglycosides is not recommended, especially when alternative agents with adequate gram negative activity are available, since they have poor lung penetration (need of high doses to achieve bactericidal concentrations in the lung), increased risk of nephrotoxicity and ototoxicity and they have been associated with poorer clinical response rate compared with other classes (1,12).
4. Acinetobacter → carbapenem or either ampicillin/sulbactam, if the isolate is susceptible to these agents. If VAP is caused by Acinetobacter species that is sensitive only to polymyxins it is recommended to use intravenous polymyxin (colistin or polymyxin B) and adjunctive inhaled colistin. The use of tigecycline and adjunctive rifampicin are not recommended.
5. Carbapenem-resistant pathogens: intravenous polymyxins (colistin or polymyxin B) and adjunctive inhaled colistin, as the latter has been associated with improvement in clinical outcomes. Colistin for inhalation should be administered promptly after being mixed with sterile water, according to the US Food and Drug Administration (FDA) (1,8,11,12).

Definitive treatment

Once the microbiology results are obtained, empirical treatment will be modified to a targeted antimicrobial therapy. The main goal for clinicians should be to avoid over-use of unnecessary broad-spectrum antibiotics, reason why all definitive therapy should be based upon the results of antimicrobial susceptibility testing and patient-specific factors (1).

First of all, if clinical suspicion is low and no pathogen is retrieved, antibiotic cessation should be considered. Secondly, in patients with bacteriologically proven VAP, antibiotics should be narrowed once microbiological results and susceptibility tests are available, including the following measures:

- Withdrawal of anti-MRSA antibiotics if no MRSA is recovered
- Restriction of carbapenems to carbapenem-only susceptible pathogens → ESBL-producing *Enterobacteriaceae* and carbapenem-only susceptible *Pseudomonas* and *Acinetobacter* spp.
- Use of narrow-spectrum agents in patients infected with susceptible strains

Last, antimicrobial therapy can be safely switched to monotherapy once pathogens responsible for infection are identified and susceptibility results have been obtained, even for *Pseudomonas* infection. Indeed, the usefulness of combination therapy is mostly to increase the likelihood of appropriateness of treatment rather than improving the prognosis of patients and therefore, once the appropriate definitive treatment has been established, the non-de-escalation of empirical treatment will only contribute to the development of multiresistances (8).

Duration of treatment

Guidelines recommend that the duration of antimicrobial treatment for VAP should not exceed nor be below 7 days in most patients (8). Longer course may be appropriate for immunocompromised patients, rare cases of empyema, lung abscess or necrotising pneumonia and pneumonias due to *Pseudomonas* or *Acinetobacter* species, as they are considered complicated and could merit at least 14 days of antibiotic therapy due to the risk of relapse associated with shorter course durations (11).

1.1.9. Prevention

Prevention of ventilator-associated pneumonia is as important as its diagnosis and management.

1. General prophylaxis for infection control

The routinary use of effective infection control measures, such as staff education, compliance with hand disinfection and isolation to reduce cross-infection with MDRO is of utmost importance, as well as surveillance of ICU infections (to identify and quantify endemic and new MDRO) and preparation of timely data to guide appropriate antimicrobial therapy.

2. Minimising exposure to invasive ventilation and lightening sedation

The prevention practices that have been associated with improving objective outcomes for ventilated patients are focused on avoiding intubation and reintubation, minimizing exposure to invasive ventilation by using non-invasive ventilation or high flow oxygen whenever feasible, as alternatives to intubation (8).

The minimisation of sedation, using spontaneous breathing trials to prompt early extubation and mobilisation may be useful to decrease the days undergoing mechanical ventilation, as well as maintaining and improving physical conditioning by providing early exercise and mobilisation.

Maintenance of ventilator circuits following the recommendations for sterilization and disinfection of respiratory care equipment should be performed to reduce the risk of circuit contamination.

3. Aspiration, body position and enteral feeding

Maintaining tracheal cuff pressure and continuous aspiration of subglottic secretions should be used, if available, to minimize pooling of secretions above the endotracheal tube cuff and prevent leakage of secretions into the lower airways, thus reducing the risk of VAP onset.

Patients should be kept in semi-recumbent position rather than supine, with a head-of-bed elevation of 30 to 45º, to prevent aspiration, especially if receiving enteral feeding. Enteral nutrition is preferred over parenteral nutrition to reduce the risk of complications related to central intravenous catheters and to prevent villous atrophy of intestinal mucosa, that may increase the risk of bacterial translocation.

4. Modulation of colonisation: topical or oral antiseptics and antibiotics

Prophylaxis with oral antibiotics (selective decontamination of the digestive tract) and regular oral care with chlorhexidine reducee the incidence of VAP, but are not recommended for routinary use, especially in patients who may be colonized with MDRO.

5. Stress bleeding prophylaxis, transfusion and hyperglicemia

Stress ulcer prophylaxis is not indicated for VAP prevention, therefore, the assessment of the need for proton-pump inhibitor and histamine-2-receptor blocker therapy should be based on the risk of gastrointestinal bleeding (2,6,11).

1.2. Film Array

Introduction to molecular microbiological diagnostics

Over the past few years, molecular methods have been developed to decrease the time between sampling organism identification and determination of antibiotic susceptibilities. This aspect is crucial to improve the clinical evolution of patients with VAP, and currently remains unsolved, being one of the main challenges in VAP diagnosis.

Conventional culture has long been the primary approach for laboratory diagnosis of lower respiratory tract infections, as it is useful in establishing definitive antibiotic therapy. Nonetheless, this technique has obvious drawbacks, since the recovery of potential pathogens may be altered due to antibiotic exposure prior to specimen collection, fastidious growth characteristics of some pathogens or overgrowth of resident flora, thus altering its sensitivity.

More importantly, standard methods currently take at least 24 to 48 hours to identify the responsible pathogen and its susceptibility to antimicrobial treatment, time that is of extreme value in critical patients with respiratory failure who require the use of mechanical ventilation.

In addition, during that time empiric broad-spectrum antibiotics are used, thus increasing the risk of multidrug-resistance acquisition with limited assurance that the causative microorganism is being covered and, in this manner, failing to meet the demand emphasized by the current American Thoracic Society (ATS) and Infectious Disease Society of America (IDSA) guidelines for management of patients with HAP or VAP (1) of reducing exposure to broad-spectrum and unnecessary antibiotics.

The recent instauration of new tools using polymerase chain reaction (PCR) to detect bacterial DNA can shorten the time of organism identification and susceptibilities, providing an alternative approach to address this problem. Some tests screen just for the main pathogens responsible for VAP and some of them also screen selected resistance mechanisms.

Furthermore, the new tools using multiplex PCR (FilmArray) directly applied to fresh samples can also be used to allow for very early de-escalation and narrowing of antimicrobial treatment in specific situations such as, for instance, the withdrawal or withholding of anti-MRSA antibiotics (8,24).

On the other hand, despite all the potential benefits that this new technique has to offer, it has to be acknowledged that FilmArray is limited by the risk of over detection (detection of DNA of non-viable organisms and detection of non-pathogenic organisms that are colonizers rather than invaders) (8,25).

This particular aspect could lead to over-use of unnecessary antibiotics, thus perpetuating the unresolved question as to whether multiplex PCR tests such as FilmArray should be used to decrease the risk of treatment failure or prevent unnecessary broad-spectrum antimicrobial therapy (26).

Further to this, it should also be noted that this technique is restricted to specific pathogens and resistance mechanisms, as it is not available to determine resistance patterns for pathogens commonly responsible for VAP such as *Pseudomonas aeruginosa*, cases in which it requires a positive culture to detect resistance mechanisms.

Film Array

The FilmArray is an emerging diagnostic method in fast detecting multiple respiratory pathogens, that consists of a multiplex polymerase chain reaction (PCR) that uses nucleic acid for the simultaneous qualitative detection and identification of multiple respiratory bacteria and viruses, as well as antimicrobial resistance genes (27).

This molecular technique requires just a few minutes of hands-on-time (2 minute manipulation) and does not require extensive knowledge of laboratory skills to be performed (no precision measurements or pipetting is required).

In addition to this, its biggest asset regarding VAP diagnosis, as mentioned above, is that it allows early organism identification and determination of antibiotic susceptibilities, having a turnaround time of about an hour, what represents an improvement of enormous magnitude in time-to-result acquisition, giving faster and exhaustive results, which may lead to better patient care (28,29).

BioFire FilmArray Pneumonia *plus* Panel

The BioFire® FilmArray® Pneumonia *plus* Panel (bioMérieux) has been selected as the best performing test in comparison with other multiplex PCR-based tests, such as Unyvero Pneumonia Panel (Curetis) (30,31).

This system allows for an automated, fast and accurate simultaneous analysis of 34 targets, 27 of which are bacteria and viruses that cause pneumonia and other respiratory tract infections, as well as 7 genetic markers of resistance to antibiotics. The FilmArray Pneumonia panel *plus* specifically analyses:

- 18 bacteria, reported into bins (10^4, 10^5, 10^6 and $\geq 10^7$ DNA copies/ml) → 11 Gram negative, 4 Gram positive and 3 atypical
- 7 antibiotic resistance markers → ESBL (CTX-M), carbapenemases (KPC, NDM, Oxa48-like, VIM, IMP) and methicillin-resistance (mecA/mecC and MREJ)
- 9 viruses (32,33)

Table 7. FilmArray® pneumonia *plus* panel targets

FilmArray® pneumonia *plus* panel targets	
15 bacteria	Reported into bins (10^4, 10^5, 10^6 and ≥10^7 DNA copies/ml)
· *Acinetobacter calcoaceticus baumanii* complex · *Enterobacter cloacae* complex · *Escherichia coli* · *Haemophilus influenza* · *Klebsiella oxytoca* · *Klebsiella pneumoniae* group · *Moraxella catarrhalis*	· *Proteus* spp. · *Pseudomonas aeruginosa* · *Serratia marcescens* · *Staphylococcus aureus* · *Streptococcus agalactiae* · *Streptococcus pneumoniae* · *Streptococcus pyogenes*
3 Atypical bacteria	· *Clamydophilia pneumoniae* · *Legionella pneumophila* · *Mycoplasma pneumoniae*
9 Viruses	· Adenovirus · Coronavirus (229E, OC43, HKU1, NL63) · human Metapneumovirus · Influenza A and B · MERS CoV · Parainfluenza viroses · Rhinovirus/Enterovirus · RSV
7 Antimicrobial resistance genes	· MRSA genes (mecA/C and MREJ) · Carbapenemases (*bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{OXA\text{-}48\text{-}like}$, *bla*$_{VIM}$, *bla*$_{IMP}$) · ESBL (*bla*$_{CTX\text{-}M}$)

It offers an overall sensitivity and specificity for BAL samples of 96.2% and 98.3%, respectively, and for sputum samples of 96.3% and 97.2% (34). The system integrates sample preparation, nucleic acid extraction and purification, amplification, detection and analysis in a single unit of equipment that requires only 2 minutes of manipulation, with a total time-to-result of approximately 1 hour (33,35).

Table 8. FilmArray® Multiplex PCR System Specifications

System Specifications	
Reagents	· Freeze-dried in durable plastic pouches · Room temperature storage
Instrument specifications	· Weight: 9kg · Size: 25.4 x 39.9 x 16.5 cm
Performance parameters	· Hands on time: approximately 2 minutes · Run turn-around time: approximately 1 hour
Environmental specification	· Operating: 15ºC to 30ºC at 20 to 80% humidity · Storage: -30ºC to 65ºC
Desktop software (Pre-loaded on supplied laptop)	· Windows-based instrument control and data analysis software · Barcode reader for data input · Automated and qualitative reporting · Separate advanced analysis software

Functioning of the FilmArray™ Multiplex PCR System

1. **Setup** → inject hydration solution and unprocessed sample into pouch

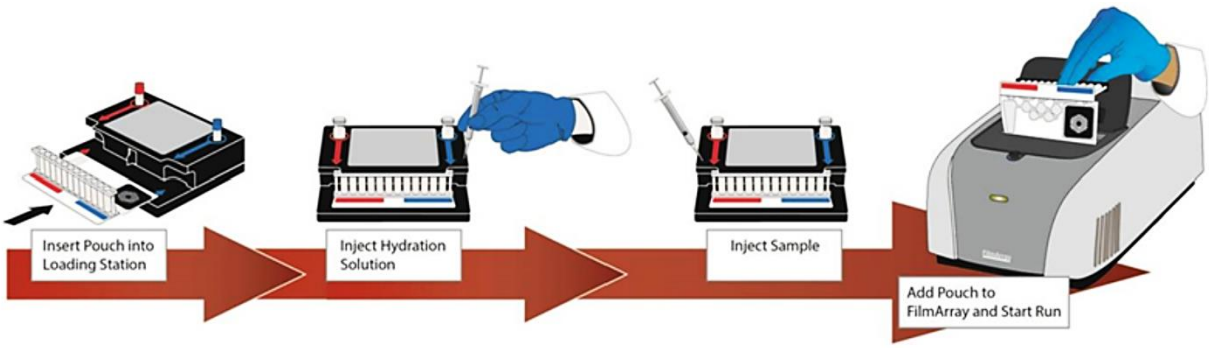
All of the reagents required to test the sample are preloaded and freeze-dried into the reagents' storage compartments of the FilmArray pouch. To begin a FilmArray run, the pouch is first placed into the loading station. Then, hydration solution is injected into the pouch through the blue inlet port on the right. The vacuum in the FilmArray pouch automatically draws the correct volume, eliminating the need for precise measuring and pipetting.

Next, sample is added to the FilmArray sample buffer. This solution is then mixed, and then injected into the FilmArray pouch through the red inlet port located on the left side of the pouch. Again, the vacuum in the pouch automatically draws the required volume.

2. **Test** → insert pouch into the FilmArray instrument and start the run

The FilmArray instrument is now ready to setup. The FilmArray pouch is loaded into the FilmArray instrument and a barcode reader is used to enter the pouch ID. The sample ID can also be scanned with the barcode reader or entered manually. Last, the user ID and password are entered and, then, the run is started. The laboratory technician is now free to walk away, since all remaining steps are completely automated. The FilmArray displays the remaining time.

Figure 2. Functioning of the FilmArray™ Multiplex PCR system. Image adapted from (33).



Inside the pouch

First, the sample is moved into the lysis chamber, where the FilmArray physically lyses any cells and viruses through a process called bead beating. Ceramic beads are agitated at high speed to break up all cells and viruses, and release the nucleic acids. These nucleic acids are then bound by magnetic beads, which are then moved from the lysis chamber to the purification chamber.

Here, a wash buffer removes any remaining cellular and viral debris. The FilmArray activates a magnet outside the pouch, which holds the magnetic beads in place while the debris is washed away. Next, an

elution buffer releases the purified nucleic acids from the magnetic beads. The magnetic beads are, again, magnetically secured while the nucleic acids are moved to the first stage PCR chamber.

A reverse-transcription step is performed to convert any target-RNA into DNA. This is followed by high order multiplex PCR involving dozens of primer pairs. During this first-stage PCR, many reactions simultaneously occur in this single large volume reaction.

The products from the first-stage PCR are then diluted to limit any remaining first-stage PCR primers. Next, the diluted first-stage PCR products are combined with a fresh mastermix and then, aliquoted to each well in the array.

Each well of this array is pre-spotted with one pair of second-stage PCR primers. These second-stage PCR primers are designed to amplify sequencies contained within the products from the first-stage PCR. This eliminates non-specific products associated with traditional multiplex PCR.

Because the second-stage primers specifically amplify only target DNA, a fluorescent double-stranded DNA binding dye is used to monitor each reaction. The second-stage primers in each well are designed to detect one specific target. Organisms are identified based on which wells in the array are positive. The FilmArray performs a melt to confirm the presence or absence of specific temperature signatures of the second-stage PCR products.

3. **Get result** → software reports whether each target is detected in the sample

The FilmArray software processes the data and makes a positive or negative call for each organism. These results are provided in one easy-to-read report (36).

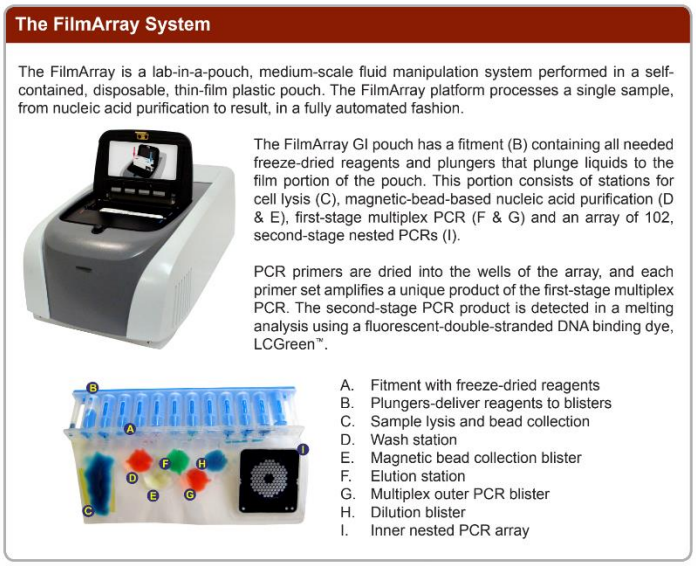


Figure 4. Functioning of the FilmArray™ System.. Image adapted from (21)

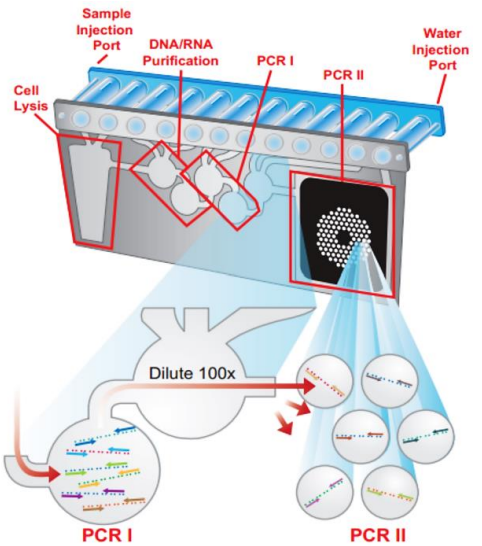


Figure 3. Functioning of the FilmArray™ panel. Image adapted from (18).

2 | Justification

Ventilator-associated pneumonia (VAP) is one of the main infectious complications in the ICU, being a significant cause of morbidity and mortality that drives the use of broad-spectrum antibiotics (26). VAP has been associated with prolonged duration of mechanical ventilation (7.6-11.5 days), ICU and hospitalization stay (11.5-13.1 days) and increased healthcare expenses, with an estimated excess cost of $20.000-40.000 per patient (1,8).

It has been estimated that VAP occurs in approximately 10% of patients undergoing mechanical ventilation, rate that has not declined over the past decade, and all-cause mortality associated with VAP has been reported to range from 20-50%, of which 13% is directly attributed to VAP (1).

Due to its potentially life-threatening condition, timely treatment is crucial to outcome in VAP, with mortality increased if antibiotics are withheld or delayed. As a matter of fact, standard methods currently take at least 24 to 48 hours to identify the responsible pathogen and its susceptibility to antimicrobial treatment.

During that time, and due to the alarmingly explosive increase in antimicrobial resistance, empiric antimicrobial treatment is prescribed with broadest-spectrum antibiotics, to overcome the fear of resistance-associated treatment failure in such critically ill and susceptible patients (10).

This counterproductive but generalized practice, then, increases the risk of multidrug-resistance acquisition with limited assurance that the causative microorganism is being covered. Intensivists are thus confronted with a permanent dilemma between the initiation of adequate antibiotic therapy and the risk of increasing MDR bacteria by the prescription of broad-spectrum antibiotics (16).

In addition to this, many clinicians frequently fail to de-escalate broad-spectrum therapy even when microbiology results become available, either unintentionally, reflecting other aspects of the patient's treatment are being prioritised, or intentionally, with clinicians reluctant to change a 'winning' therapy so that the patient does not deteriorate again (10)

Over the past few years, molecular diagnostic methods, such as FilmArray rapid multiplex PCR, have been developed to offer a potential route to overcoming these limitations by decreasing the time between sampling organism identification and determination of its antimicrobial susceptibilities, exponentially improving the turnaround time by achieving results in hours instead of days (8).

Nevertheless, as the use of this technique is only emerging, few data exist on whether it improves antimicrobial stewardship and there is no data on whether it offers advantages regarding clinical outcomes of patients (10).

The aim of this study is to determine if routine application of FilmArray on VAP diagnosis allows for immediate targeted treatment or, at least, much earlier therapeutic refinement, reducing as well the burden of multidrug-resistant organisms acquisition and therefore meeting the efforts to improve antimicrobial stewardship and clinical outcomes of patients diagnosed with VAP.

3 | Hypothesis

Molecular testing (FilmArray) provides results that are highly consistent with those of the standard of care technique, conventional culture, and allows an early start of appropriate targeted antibiotic treatment, improving the clinical outcome of patients.

4 | Objectives

4.1. Main objectives

The main objective of the first phase of the study is to evaluate the accuracy of molecular testing (FilmArray) in comparison with the standard of care technique (conventional culture) in the diagnosis of ventilator-associated pneumonia to determine if there is concordance between both techniques in pathogen detection (congruence or disparity, superiority or inferiority).

The main objective of the second phase of the study is to determine if the use of molecular testing (FilmArray) in the diagnosis of ventilator-associated pneumonia allows an early start of appropriate targeted antibiotic treatment, improving the clinical outcome of patients.

4.2. Secondary objectives

- To assess if conventional culture has provided in any case relevant information in comparison with FilmArray.
- To compare the differences in clinical outcomes between participants treated according to FilmArray molecular results versus those treated according to conventional culture (decrease in ICU length of stay, septic shock rates or mortality rates)
- To determine if there is a difference in total antibiotic use and ventilator-free days between the two groups.

5 | Material and methods

5.1. Study design

This will be a single-centre study exploring the potential impact of FilmArray rapid molecular techniques in the diagnosis and treatment of VAP, aiming for superiority in clinical outcomes of pneumonia and with regard to antimicrobial stewardship, compared with routine diagnostic methods.

The study will be carried out in two phases: a first phase of retrospective analysis of microbiological data and a second phase of prospective study of the potential impact of FilmArray rapid molecular techniques in the diagnosis and treatment of ventilator-associated pneumonia.

1. **First phase**: Cross-sectional Retrospective Observational study using collected data from the ICU

The first phase of the study consists of doing a retrospective analysis of the data that has been collected from the ICU, to determine if the results of molecular testing (FilmArray) are consistent with those of the standard care technique, conventional culture, in the diagnosis of VAP.

For this phase, 66 samples from patients diagnosed with VAP have been selected from the ICU of Santa Caterina Hospital (IAS) through consecutive sampling. The participants have provided lower respiratory tract samples from bronchoalveolar lavage fluid (BAL) or endotracheal aspirate.

Each sample has been immediately sent to be analysed, in parallel, through two methods: conventional culture and FilmArray pneumonia plus panel. With this being done, the first phase of this study will consist in assessing the obtained results to determine:

- If the results obtained with the FilmArray pneumonia panel are consistent with those of the conventional culture (congruence or disparity in pathogen detection, superiority/inferiority of detection of one technique over the other)
- If the culture has provided in any case relevant information in comparison with FilmArray

2. **Second phase**: Prospective, Open-labelled, Single-Centre, Randomised Controlled Clinical Trial

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The second phase of the study consists of conducting prospective randomised controlled clinical trial to determine if the use of FilmArray allows an early start of appropriate directed antimicrobial treatment, favouring the patient's clinical evolution, without over-treating him.

For this phase, the recruited patients will be randomly divided into two groups, through a computer-based randomization statistical software (SPSS) that will generate a specific identification patient number for the study and will perform a randomization of the patients, assigning them in a ratio 1:1 to the control group or the group of intervention.

Control group

In the participants assigned to the control group, lower respiratory tract samples will be obtained from bronchoalveolar lavage fluid (BAL) or endotracheal aspirate and will be sent to study with the standard of care technique, the conventional culture.

While awaiting culture results, empiric antimicrobial therapy will be administered, which will be continued until culture results are available. If culture results suggest it, empiric antimicrobial treatment will be modified to tailor it to the specific pathogen, replacing it for a targeted antimicrobial treatment. For each patient, the clinical evolution will be assessed.

Intervention group

In the participants assigned to the intervention group, lower respiratory tract samples will be obtained from bronchoalveolar lavage fluid (BAL) or endotracheal aspirate (BAS) and will be sent to study with the intervention plus the standard of care → FilmArray multiplex PCR + conventional culture.

The FilmArray® Pneumonia *plus* Panel detects 18 bacteria and 9 viruses, as well as 7 genetic markers of resistance. The analyser units will be located on the clinical Laboratory of Microbiology and Parasitology of Santa Caterina Hospital. The antimicrobial therapy will be directed based on the results obtained with the FilmArray Pneumonia plus panel, according to the established recommendations of most recent guidelines. For each patient, the clinical evolution will be assessed.

5.2. Study population

The population of this study will consist in critically ill adults (>18 years of age) diagnosed with ventilator-associated pneumonia (VAP) admitted to the Intensive Care Unit of Hospital Santa Caterina.

Inclusion criteria

- 18 or more years of age
- Diagnosed with ventilator-associated pneumonia being treated in the Intensive Care Unit
- Able to provide sufficient volume of airway specimen obtained for routine testing and FilmArray

Exclusion criteria

- Requires antibiotic treatment for indications other than VAP
- Expected to die within 2 days due to underlying disease
- Have an existing directive to withhold life-sustaining treatment, in relation to antibiotic use
- Determined to be unfit by the study investigator

5.3. Sample

5.3.1. Sample selection

All patients included in the study will be recruited from the Intensive Care Unit (ICU) of Santa Caterina Hospital (Girona). Patients diagnosed with VAP who meet the inclusion criteria will be considered eligible to participate in the study.

First phase

For the first phase, a total of 66 microbiologic samples (previously selected through consecutive sampling) are available, to whom both techniques have been performed, resulting in 132 samples available for analysis (66 culture samples + 66 FilmArray samples).

Second phase

For the second phase, sample recruitment will be performed through consecutive sampling as well, expecting a response rate of 90% of the patients considering they will all be in-patients treated in the Intensive Care Unit. Patients diagnosed with VAP who meet the inclusion criteria will be considered eligible to participate in the study.

5.3.2. Sample size

For the retrospective phase of the study, the sample size will be the total number of samples collected in the Intensive Care Unit up to this point, which currently stands at 66.

For the prospective phase of the study (2nd phase), the sample size has been calculated with the help of the 'GRANMO Sample Size Calculator'.

In view of the literature published so far, the proportion of patients receiving appropriate antibiotics in the group studied with conventional culture is 53%. Considering that we want to improve this by at least 20%, the expected proportion of patients receiving appropriate antibiotics in the group studied with FilmArray would be 73%.

Accepting an alpha risk of 0,05 and a beta risk of 0,2 in a two-sided test, it has been estimated that a number of 100 subjects in the intervention group and 100 subjects in the control group will be required to find a statistically significant difference in proportion, which is expected to be 0.53 in group 1 and 0.73 in group 2. A dropout rate of 10% (0.1) has been predicted.

5.3.3. Estimated time for sample recruitment

Considering that pneumonia generally follows a seasonal pattern, being more frequent in spring and winter, the patient recruitment period will include, at least, a 12-month period with all seasons of the year, in order to detect any seasonal trend.

Taking into account that the collected epidemiological data estimate that the reported cases of VAP are approximately 75 cases per year, the estimated time to reach the sample size of the study should be about 2,6 years.

5.3.4. Randomisation and masking

As previously mentioned before, the recruited patients will be randomly divided into two groups, through an electronic randomization statistical software that will generate a specific identification patient number for the study and will perform a randomization of the patients.

Given the nature of the study, it is not possible to conduct a blinded trial, as hospital ICU and laboratory clinicians must be aware of the assigned intervention in order to properly manage the patients and conduct their clinical practice. For that reason, once a participant is randomized, the trial is open-label, meaning clinicians and all study team members will know which group a participant is in.

To prevent or reduce the bias associated to this kind of studies and achieve objective outcomes, independent clinicians who are not involved in the trial will act as outcome assessors, will remain blinded to the assignations and under no circumstances allocation will be revealed to them.

5.4. Variables

5.4.1. Study variable

The study (independent) variable is the application of FilmArray in the diagnosis of ventilator-associated pneumonia in order to administer an appropriate early targeted antimicrobial treatment. Appropriate antibiotic is defined as active (receiving an antimicrobial active against the organism(s) detected in vitro) and proportionate (not excessively broad spectrum for the pathogen(s) identified).

5.4.2. Outcome variable

Primary outcomes

1. *Change in SOFA score* (Sequential Organ Failure Assessment) from randomisation to 7-days. The existence of organ dysfunction is identified by a variation of 2 or more points on SOFA scale.
2. *Duration of mechanical ventilation*: number of days undergoing mechanical ventilation
3. *Hospital and ICU length of stay*: time from randomisation to discharge
4. *30-day mortality*: death of any cause within 30 days since randomisation

Secondary outcomes

- *Patients with an identified etiological agent*
- *Patients whose antimicrobial treatment is modified*: presence or absence of modification of antimicrobial therapy, time until modification and cause (escalation, de-escalation)
- *Duration of the entire antibiotic treatment:* total-per-patient antibiotic usage defined as number of days that the patient was administered with antibiotics for the treatment of VAP
- *Acquisition of MDRO during hospital stay*: multi-drug resistance is defined as in vitro resistance against 1 or more agents in 3 or more antibiotic classes
- *Positivity of other cultures* (blood cultures, urinalysis)
- *Number of antibiotic-related adverse events: Clostridium difficile* colitis or adverse drug effects

5.4.3. Covariables

1. Age (years)
2. Gender (female or male)
3. Severity → APACHE II score at ICU admission (**Annex 1**)
4. Risk factors for MDRO (**TABLE 2**)

Table 9. Description of the variables included in the study

	Variable	Type	Category of values
Study variable	Application of FilmArray	Dichotomic qualitative	Yes / No
Primary outcome variables	Change in SOFA score	Dichotomic qualitative	Improvement / Equality or worsening
	Duration of mechanical ventilation	Quantitative	Numerical (days)
	Hospital and ICU length of stay	Quantitative	Numerical (days)
	30-day mortality	Dichotomic qualitative	Alive / Deceased
Secondary outcome variables	Patients with an identified etiological agent	Dichotomic qualitative	Yes / No
	Duration of entire antimicrobial treatment	Quantitative	Numerical (days)
	Modification of treatment		
	Patient with a modification	Dichotomic qualitative	Yes / No
	Time until modification	Quantitative	Numerical (days)
	Cause of the modification	Dichotomic qualitative	Escalation / De-escalation
	Acquisition of MDRO	Dichotomic qualitative	Yes / No
	Positivity for other cultures (blood, urine)	Dichotomic qualitative	Yes / No
	Number of antibiotic-related adverse events	Quantitative	Numerical
Covariables	Age	Discrete quantitative	Numerical (years)
	Gender	Dichotomic qualitative	Female Male
	APACHE II score at ICU admission	Quantitative	Numerical
	Risk factors for MDRO	Dichotomic qualitative	Yes / No

Abbreviations → MDRO: Multi-drug resistant organism // SOFA: Sequential Organ Failure Assessment // ICU: Intensive Care Unit // APACHE: Acute Physiologic and Chronic Health Evaluation

5.5. Statistical analysis

The research team of the study will perform the statistical analysis using IBM Statistical Package for the Social Sciences (SPSS) software. For all analyses, 95% confidence interval will be taken, and the results will be considered statistically significant when the p-value is ≤0.05. Analysis will be made by intention to treat (ITT).

5.5.1. Descriptive analysis

First of all, a descriptive analysis of all the variables will be performed and included in a table, defining them as quantitative or qualitative.

Retrospective phase

For the retrospective phase a **concordance analysis** between FilmArray and conventional culture will be done. In particular, the main interest of this phase of the study is to analyse if pathogen detection is equal or different in both techniques. The analysis will be done for each pathogen (presence/absence) by using the Cohen's kappa.

Prospective phase

For the prospective phase, **quantitative variables** (duration of mechanical ventilation, hospital and ICU length of stay, for the primary objectives; and time taken to modify antimicrobial therapy, duration of the entire antibiotic treatment and number of adverse events for the secondary objectives) will be summarized using medians and interquartile range.

Qualitative variables (change in SOFA score and 30-day mortality for the primary objectives; and identified etiological agent, modification of treatment, cause of modification, acquisition of MDRO and positivity of other cultures, for the secondary objectives) will be summarized using percentages.

Once this is done, all these analyses will be stratified by the application of FilmArray and conventional culture, and an additional stratification will be done by the covariates. **Age and severity** will be categorized in quartiles.

Kaplan-Meier curves of duration of mechanical ventilation, hospital and ICU length, time taken to modify antimicrobial therapy and duration of the entire antibiotic treatment, **duration variables** hereinafter, will be estimated and drawn. As above, these curves will be stratified by the application of FilmArray and conventional culture and, secondly, by the covariates.

5.5.2. Bivariate inference

For the prospective phase of the study, to determine whether there is a correlation between the variables, a bivariate analysis will be performed. The difference of the medians of the **quantitative variables** will be tested by using the Mann-Whitney's U test.

The difference of proportions of the **qualitative variables** will be contrasted using the chi-square (x^3) test or, in case that the expected number of counts in one cell is lower than 5, Fisher's exact test. For **duration variables** the difference between Kaplan-Meier curves will be assessed with Log-Rank test.

5.5.3. Multivariate analysis

In order to establish the statistical association between the variables of the study, while controlling the effect of possible confounding factors or effect modifiers, a multivariate analysis will be performed.

With the aim of studying this statistical significance, Poisson regression will be used to assess the effect of the intervention on the **quantitative variables**, while controlling for the covariates, and logistic regression will be used to assess the effect of the intervention on the **qualitative variables**, again controlling for the covariates.

For **duration variables**, the assessment will be made by means of Cox regression, adjusting for covariates.

5.6. Data collection

For the first phase (retrospective microbiological analysis), data collection has already been carried out by clinicians of the ICU of Santa Caterina Hospital, who have kept the results of the samples of respiratory patients diagnosed with VAP on whom both conventional cultures and FilmArray were performed in parallel with the aim of conducting a subsequent concordance analysis.

For the second phase, the research team will create a computer-based database using IBM Statistical Package for the Social Sciences (SPSS) software for the collection of data. Although the clinical trial is open-labelled and clinicians will know which group each participant is in, each patient will be given a specific identification number for the study and all personal data will be codified to respect anonymity as much as possible.

The data that will be included in the database regarding the patient's clinical evolution will be extracted from Centricity^{TM} High Acuity Critical Care platform, which contains a complete record of the medical data of the patients: clinical data from equipment measurements, diagnostic tests and clinical history.

Moreover, a record will be kept of the data specified in the data collection form (**Annex 4**), which will have to be filled in for each patient included in the study and subsequently added in the study database.

6 | Ethical aspects and legal considerations

This study will be conducted in compliance with the latest revision of the Declaration of Helsinki for Ethical Principles for Medical Research Involving Human Subjects (last updated in October 2013).

Before the start of the research, this protocol will be submitted for review by the Clinical Research Ethics Committee (CEIC) of Girona. Once the approval is received, the project will begin.

The four basic ethical principles of Beauchamp and Childress will be respected as follows:

1. Autonomy: patients autonomy is going to be respected according to the "Ley 41/2002, de 14 de noviembre, básica reguladora de la autonomía del paciente y de derechos y obligaciones en materia de información y documentación clínica".

A key ethical consideration of this study is that patients in ICUs often lack the capacity to consent for themselves and the clinical trial is going to be conducted in an emergency setting, where consent cannot reasonably be taken before intervention. The aims of the trial are incompatible with only entering patients with capacity and who are able to consent quickly, as it is important to ensure that the findings can be generalised to clinical practice.

This study does not aim to study an invasive treatment of medicine, the same antibiotics and decisions will be available to clinicians at all times and they may choose to disregard the FilmArray results for antimicrobial guidance if it is clinically indicated.

Therefore, considering the emergency setting where the study will take place, informed consent to participate will be obtained by representation (37), which means that the decision of the patient, unable to formulate it for medical reasons, will be replaced by that of another person who will decide in his place. The representative shall be that person related to him, for family or *de facto* reasons.

In cases where consent by representation cannot be obtained, due to lack of representative or for reasons of incapacity of the same, informed consent will be obtained retrospectively, as soon as reasonably appropriate from all patients or their legal proxies.

Once aware of their inclusion in the study, participants and their legal proxies will be given as long as needed to make an informed decision and the participant will remain in the trial whilst a decision is sought and as long as there is no objection. Any participant that wishes to withdraw their inclusion in the study will be excluded from the final sample analysis to ensure their autonomy to decide.

In most cases, it is anticipated that the patient's consultee will be approached to give assent due to the expected incapacity of most ICU patients to give consent. Should the participant recover from their life-threatening condition and regain capacity, they will be approached about the study directly and, in this circumstance, their consent or refusal will override the consultee's agreement.

Additionally, all participants or their legal proxies will be given a written information sheet and a consent form where details of the study will be extensively explained. These documents will be submitted in advance to the ethics committee for formal approval (**Annexes**).

2. Non-maleficence: patients who meet the exclusion criteria will be excluded from the project, as they would not benefit from the study procedure. At all times, clinicians may choose to disregard the FilmArray results for antimicrobial guidance if it is clinically indicated, considering that in these situations it is of utmost importance to make no harm.
3. Beneficence: the inclusion criteria have been described with the intention of including patients who will benefit most from the study procedure. Moreover, the therapeutic management will not be inferior in any patients, as they will be treated with the same available antimicrobials than population not included in the study.
4. Justice: all the patients who are eligible for the study will be considered equally for participation in the study, ensuring fairness and equality among individuals.

The confidentiality of the participants will be preserved by anonymizing the collected data, assigning a specific identification patient number for the study.

All data obtained will be entered and processed in a database to which only the research team will have access, and protection of personal data will be conducted according to "Ley Orgánica 3/2018, de 5 de diciembre, de Protección de Datos Personales y garantía de los derechos digitales" and "Reglamento (UE) 2016/679 del Parlamento Europeo y del Consejo, de 27 de abril de 2016, relativo a la protección de las personas físicas en lo que respecta al tratamiento de datos personales y a la libre circulación de estos datos".

Last of all, all investigators will have to declare that the research will be conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest to guarantee that there is no commercial bias.

7 | Working plan and chronology

This study will be carried out by a research team composed of the following:

- General coordinators: Mireia Jurado and Miquel Morales will direct all phases of the research and will pool the necessary data from the hospital.
- Sponsorship: the general coordinators of the study will be responsible for the expenses incurred by the clinical trial, with the financial support of public funding that will be sought trough calls for research project grants opened by the Economy Department of the Ministry. Private funding, through commercials, will only be accepted if no potential conflict of interest can arise from it.
- Hospital clinicians (ICU and laboratory personnel): ICU physicians and laboratory personnel will not intervene in the final outcome assessment but will, however, be a part of the research team, as they will be the ones responsible for the in-hospital management of patients.
- Team of investigators
  - Principal investigator: one of the study investigators will be designed as main investigator, who will be ultimately responsible for the overall research and design, supervising the project and elaborating the results of the final report, writing conclusions extracted by outcome assessors.
  - Co-investigators: the group of coinvestigators will be composed of the outcome assessors of the study, who will remind blinded to avoid biases and preserve anonymity.
- Professional statistician: a qualified statistician from Institut d'Investigació Biomèdica de Girona (IdIBGi) will be hired to carry out data collection and statistical analysis.

The estimated time of intervention will be 4 years and will include 4 main phases. Throughout the study period, periodic follow-up meetings will be scheduled for all the research team personnel.

Phase 0: Protocol elaboration

September 2022 to December 2022

- Recruitment of personnel for the research team
- Bibliographic search: literature and background review
- Drafting and presentation of the protocol
- Ethical evaluation of the ethics committee
- Explanation of the study and distribution of tasks

Phase 1: Intervention

Phase 1A: Retrospective analysis

January 2023 to March 2023

- Retrospective analysis of the 66 samples
- Preparation of tables and graphics and interpretation of results

Phase 1B: Prospective phase

January 2023 to June 2025

- Patient recruitment. All eligible patients will be adequately informed and given a consent form
- Randomisation of patients to control or intervention group and application of the study design
- Periodic meetings of the general coordinators and study investigators

Phase 2: Statistical analysis

June 2025 to September 2025

- Meeting with the entire research team
- Data collection and development of an anonymized database
- Univariate, bivariate and multivariate data analysis
- Preparation of tables and graphics, outcome assessment and interpretation of results

Phase 3: Final report

September 2025 to December 2025

- Possible modifications and drafting of the final report
- Presentation of the final report to the scientific community
- Participation in national congress of intensive care unit

A chronogram of the working plan is presented in **TABLE 10**.

Table 10. Chronogram of the working plan

Study phases	2022				2023			2024			2025											
	09	10	11	12	01 – 04	05 – 08	09 – 12	01 – 04	05 – 08	09 – 12	01	02	03	04	05	06	07	08	09	10	11	12
Stage 0																						
Bibliographic search																						
Protocol elaboration																						
Ethical evaluation																						
Stage 1A - Retrospective analysis																						
Sample analysis																						
Results interpretation																						
Stage 1B. Prospective study																						
Sample recruitment and data collection																						
Stage 2. Statistical analysis																						
Data compilation																						
Statistical analysis																						
Stage 3. Final report																						
Drafting																						
Publication																						

*The numbers correspond to the months of the year, being 1 - January, 2 - February, 3 - March, 4 - April, 5 - May, 6 - June, 7 - July, 8 - August, 9 - September, 10 - October, 11 - November and 12 - December.

8 | Budget

8.1. Personnel costs

- Hospital clinicians participating in the research team will perform their duties as part of their work activity, reason why they will not be economically rewarded for their involvement in the project, as they will monthly receive their corresponding salary from the hospital.
- Professional statistician from IdIBGi, who will be hired to work an approximate number of 40 hours for the retrospective analysis plus 100 hours for the prospective phase, making a total sum of 140 hours. Expecting a price per hour of 30€ the final cost will be of approximately 4.200€.
- Bearing in mind that the intervention that will be performed on patients will be minimal and is already being used in routine clinical practice, an insurance policy will not be required.

8.2. Material costs

- FilmArray equipment → the hospital in this study already owns the necessary equipment to perform a FilmArray multiplex PCR pneumonia *plus* panel, which has an approximate cost of 17.500€ (shipment included) so, therefore, it is not necessary to include this cost in the final price.
- Conventional culture preparation equipment → the hospital in this study already owns the necessary equipment to perform conventional cultures, as it is their routine diagnostic method for many pathologies. The equipment has an approximate cost of 2.000€ (315€ for 200 culture plates and 1635€ for a culture growing stove plus 50 additional euros for preparation material).
- Printing materials → for the patient's study information sheet and informed consent we will print a total of 400 pages (2 pages x 200 patients), with 0,05€/page, being the final cost of 20€.

8.3. Divulgation costs

- Publication expenses → 1.000€ per publication will be required to publish an article in a journal
- Inscription for congress → the inscription of attendance to the national congress of Intensive Care Medicine has an approximate cost of 520€ per attendant.

The total costs of the study are summarized in **TABLE 11**.

Table 11. Total estimated budget of the study.

Item	Quantity	Price per unit (€)	Total price (€)
Personnel costs			
Outcome assessors	2 assessors, 2 months, 30h per month	30,00€/h	3.600€
Statistician	40 hours + 100 hours	30,00€/h	4.200€
Materials and tests			
Printing costs	400 pages	0,05€/page	20,00€
FilmArray equipment	1 thermocycler	17.500€	(already owned) 0,00€
Conventional culture preparation equipment	200 culture plates + 1 growing stove	1,57€/plate 1.635€/stove	(already owned) 0,00€
Divulgation costs			
Publication expenses	1 publications	2.000€	2.000€
Inscription for congress	2 inscriptions	520,00€ per attendant	1.040€
			10.800€

9 | Study strengths and limitations

The present study has several strengths and limitations that have to be acknowledged and are exposed as follows:

Single centre study

To begin with, the fact that the study is single-centred entails a series of advantages, among which is that it is logistically simpler, more economical, does not require lengthy negotiations on the study protocol and usually deals with a less heterogeneous population, thereby reducing confounding.

These sort of studies, however, have limited internal validity (and, therefore, limited external validity as well), so their broadly implementation may be based on flawed results and should be taken into consideration when extracting conclusions.

Further to this, another limitation that we can encounter in single-centre studies is the unequal allocation of resources, in the sense that what may be easily applicable in a centre with loads of resources and experience, may be more complicated to apply in a centre with limited ones.

In spite of this, these limitations are common in single-centre critical care studies, which provide an essential starting point for testing interventions and then allow larger multicentre studies to be properly planned and powered, and could be overcome by conducting a multicentric study.

Study length

This study protocol has estimated a time for patient recruitment of 2,6 years, making the expected duration of the project of about 4 years.

Bearing in mind that FilmArray is an emerging technique that is attracting increasing interest in the scientific community, there is a chance that, throughout the time of study intervention, a new article may appear that establishes the FilmArray as the new Gold Standard technique for VAP diagnosis, thus invalidating the methods and main purpose of our study.

This limitation stands on the same basis as the previously mentioned one, which could also be solved by performing a multicentric study, thus reducing the estimated time for patient recruitment and study intervention.

Open-label study

Given the nature of the study, as well as many other studies that are conducted in Intensive Care Units, the lack of blinding is inevitable, as clinicians need to be aware of the assigned intervention in order to properly guide antimicrobial therapy and adequately manage patients. This has to be taken into account, as it increases the possibility of detection bias.

To prevent or reduce this bias and obtain objective outcomes, this study will have independent physicians who are not involved in the trial to act as outcome assessors, who will remain blinded to the assignations and under no circumstances will the allocation be disclosed to them.

Consecutive sampling

The sample selection of this study has been done with a non-probabilistic method, by using consecutive sampling. This inherently associates the possibility that there is a selection bias, which should be taken into account when considering the results of the study.

Limitations of the technique

The limitations of this study should also acknowledge the limitations of the study technique, since FilmArray is limited by the risk of over detection, due to detection of DNA of non-viable organisms and detection of non-pathogenic organisms that are colonizers rather than invaders.

Further to this, it should also be noted that this technique is restricted to specific pathogens and resistance mechanisms, as it is not available to determine resistance patterns for pathogens commonly responsible for VAP such as *Pseudomonas aeruginosa*, cases in which it requires a positive culture to detect resistance mechanisms.

This particular aspects should be considered as possible misleading factors when interpreting the results of the study or when trying to prove the potential impact of FilmArray in ventilator-associated pneumonia, in comparison with standard-of-care testing with conventional culture.

10 | Clinical and healthcare impact

Ventilator-associated pneumonia (VAP) is one of the main infectious complications in patients admitted to intensive care units, being one of the leading causes of antibiotic use and a significant cause of morbidity and mortality.

Due to its potentially life-threatening condition, timely treatment is crucial to outcome in VAP, with mortality increased if antibiotics are withheld or delayed. As a matter of fact, standard methods currently take at least 24 to 48 hours to identify the responsible pathogen and its susceptibility to antimicrobial treatment.

During that time, and due to the alarming exponential increase in antibiotic resistance, empiric antimicrobial treatment is prescribed with broadest-spectrum antibiotics, to overcome the fear of resistance-associated treatment failure in such critically ill and susceptible patients.

This counterproductive but generalized practice, then, increases the risk of multidrug-resistance acquisition with limited assurance that the causative microorganism is being covered.

Over the past few years, molecular diagnostic methods, such as FilmArray rapid multiplex PCR, have been developed to decrease the time between sampling organism identification and determination of antibiotic susceptibilities, exponentially improving the turnaround time by achieving an automated display of results in approximately 1 hour.

In such a way, this technique may have a significant effect in the management of this critically ill patients, offering an alternative to address this issue by obtaining early pathogen identification and associated multiresistances, as well as by improving antimicrobial stewardship, since:

- It will allow early pathogen identification, therefore advancing the opportunity to match the antibiotic to the present pathogen
- It will determine its antibiotic susceptibilities, therefore reducing the need of prescribing broad-spectrum empirical treatment and decreasing as well the development of multidrug resistance.
- It may allow early targeted antimicrobial treatment, decreasing the consumption of broad spectrum antibiotics both by limiting their prescription and shortening their duration

In addition to all this, the characteristics of FilmArray may offer some additional benefits in comparison with standard of care technique:

On the one hand, if FilmArray resulted to have superiority in pathogen detection as compared to conventional, culture, the identification of a specific pathogen could enable modification of empirical antibiotic therapy (escalation or de-escalation), even in the face of a negative culture.

On the other hand, as results of bacteria detection will be expressed in a quantitative manner, detection of a clinically significant high pathogen concentration could potentially prevent the early termination of effective antibiotics, as well as it could allow appropriate narrowing or discontinuation of antibiotic therapy if pathogen concentration were too low to be considered as responsible of the infection.

Besides this, the detection of the abundance of each organism in a polymicrobial specimen could be of value to target therapy to the most likely pathogen.

Considering that the role of viral pathogens in VAP has only recently been appreciated, in part due to the increased availability of multiplex molecular panels to detect these agents, the recent acknowledgement of its virulence due to the ongoing pandemic and the lack of availability of an effective therapy for this etiology; it has to be taken into account that their role in VAP may have been underestimated and, therefore, underdiagnosed.

This uncertainty may be solved by the simultaneous detection of the most common targets that FilmArray multiplex PCR offers, being another potential contribution to VAP diagnosis.

In view of the potential impact of FilmArray, for the reasons that have been mentioned above, it can be concluded that its routinely application on VAP diagnosis deserves to be considered, not as a replacement for conventional culture, but rather as an adjunctive test to provide rapid identification of the etiological diagnosis, antibiotic susceptibilities of the responsible organism and, furthermore, to positively impact on antimicrobial stewardship and clinical improvement in patients' outcomes.

11 | Bibliography

1. Kalil AC, Metersky ML, Klompas M, Muscedere J, Sweeney DA, Palmer LB, et al. 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 Sep 1;63(5):e61–111.

2. American Thoracic Society, Infectious Diseases Society of America. Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. Am J Respir Crit Care Med. 2005 Feb 15;171(4):388–416.

3. Sousa AS, Ferrito C, Paiva JA. Intubation-associated pneumonia: An integrative review. Intensive Crit Care Nurs. 2018 Feb;44:45–52.

4. Hayashi Y, Morisawa K, Klompas M, Jones M, Bandeshe H, Boots R, et al. Toward Improved Surveillance: The Impact of Ventilator-Associated Complications on Length of Stay and Antibiotic Use in Patients in Intensive Care Units. Clin Infect Dis. 2013 Feb 15;56(4):471–7.

5. Klompas M. Ventilator-Associated Conditions Versus Ventilator-Associated Pneumonia: Different by Design. Curr Infect Dis Rep. 2014 Oct 1;16(10):1–7.

6. Klompas M, Branson R, Eichenwald EC, Greene LR, Howell MD, Lee G, et al. Strategies to prevent ventilator-associated pneumonia in acute care hospitals: 2014 update. Infect Control Hosp Epidemiol. 2014 Aug;35(8):915–36.

7. Patil HV, Patil VC. Incidence, bacteriology, and clinical outcome of ventilator-associated pneumonia at tertiary care hospital. J Nat Sci Biol Med. 2017;8(1):46–55.

8. Papazian L, Klompas M, Luyt CE. Ventilator-associated pneumonia in adults: a narrative review. Intensive Care Med. 2020 May;46(5):888–906.

9. Sociedad Española de Medicina Intensiva Crítica y Unidades Coronarias (SEMICYUC). ENVIN - HELICS [Internet]. 2021 [cited 2022 Nov 3]. Available from: https://hws.vhebron.net/envin-helics/

10. High J, Enne VI, Barber JA, Brealey D, Turner DA, Horne R, et al. INHALE: the impact of using FilmArray Pneumonia Panel molecular diagnostics for hospital-acquired and ventilator-associated pneumonia on antimicrobial stewardship and patient outcomes in UK Critical Care—study protocol for a multicentre randomised controlled trial. Trials. 2021 Oct 7;22:680.

11. Rae Modi A, Kovacs CS. Hospital-acquired and ventilator-associated pneumonia: Diagnosis, management, and prevention. Cleve Clin J Med. 2020;87(10):633–9.

12. Torres Martí A, Rodríguez de Castro F. Infecciones respiratorias. In: Farreras Valentí P, Domarus A von, Rozman C, Cardellach F, editors. Medicina interna. 19th ed. Barcelona: Elsevier; 2020. p. 711–24.

13. Ortiz G, Dueñas C, Garay M. Neumonía asociada a la ventilación mecánica: prevención, diagnóstico y tratamiento. Acta Colomb Cuid Intensivo. 2015 Oct;15(4):312–21.

14. Chi SY, Kim TO, Park CW, Yu JY, Lee B, Lee HS, et al. Bacterial Pathogens of Ventilator Associated Pneumonia in a Tertiary Referral Hospital. Tuberc Respir Dis. 2012 Jul 31;73(1):32–7.

15. Tetaj N, Capone A, Stazi GV, Marini MC, Garotto G, Busso D, et al. Epidemiology of ventilator-associated pneumonia in ICU COVID-19 patients: an alarming high rate of multidrug-resistant bacteria. J Anesth Analg Crit Care. 2022 Aug 19;2(1):36.

16. Peiffer-Smadja N, Bouadma L, Mathy V, Allouche K, Patrier J, Reboul M, et al. Performance and impact of a multiplex PCR in ICU patients with ventilator-associated pneumonia or ventilated hospital-acquired pneumonia. Crit Care Lond Engl. 2020 Jun 19;24(1):366.

17. Asensio Martín MJ, Hernández Bernal M, Yus Teruel S, Minvielle A. Infecciones en el paciente crítico. Medicine (Baltimore). 2018 Apr;12(52):3085–96.

18. Bruder E, Ball I, Ridi S, Pickett W, Hohl C. Etomidato para sedar a pacientes graves durante la intubación endotraqueal de urgencia. Cochrane Database Syst Rev. 2015;(1):CD010225.

19. Ego A, Preiser JC, Vincent JL. Impact of Diagnostic Criteria on the Incidence of Ventilator-Associated Pneumonia. Chest. 2015 Feb;147(2):347–55.

20. Torres A, Niederman MS, Chastre J, Ewig S, Fernandez-Vandellos P, Hanberger H, et al. International ERS/ESICM/ESCMID/ALAT guidelines for the management of hospital-acquired pneumonia and ventilator-associated pneumonia. Eur Respir J. 2017 Sep;50(3):1700582.

21. Leone M, Bouadma L, Bouhemad B, Brissaud O, Dauger S, Gibot S, et al. Brief summary of French guidelines for the prevention, diagnosis and treatment of hospital-acquired pneumonia in ICU. Ann Intensive Care. 2018 Nov 3;8(1):104.

22. Sicilia-Urbán JJ, Sanz-Rojas P, Serrano-Heranz R. Protocolo diagnóstico de la neumonía adquirida en la comunidad y neumonía nosocomial. Medicine (Baltimore). 2010 Mar 1;10(50):3389–93.

23. Aldea Mansilla C, Gracia Ahufinger I, Guembe Ramírez M, Martínez Alarcon J. 15a. Diagnóstico microbiológico de las infecciones asociadas a catéteres intravasculares. In: Mansilla EC, Moreno RC, editors. Procedimiento de Microbiología Clínica. Madrid: SEIMC; 2018. p. 1–50.

24. Marimón JM, Navarro-Marí JM. Métodos de diagnóstico rápido de las infecciones respiratorias. Enferm Infecc Microbiol Clin. 2017 Feb;35(2):108–15.

25. Poritz MA, Blaschke AJ, Byington CL, Meyers L, Nilsson K, Jones DE, et al. FilmArray, an Automated Nested Multiplex PCR System for Multi-Pathogen Detection: Development and Application to Respiratory Tract Infection. PLoS ONE. 2011 Oct 19;6(10):e26047.

26. Guillotin F, Poulain C, Gaborit B, Bouras M, Cinotti R, Lakhal K, et al. Potential Impact of Rapid Multiplex PCR on Antimicrobial Therapy Guidance for Ventilated Hospital-Acquired Pneumonia in Critically Ill Patients, A Prospective Observational Clinical and Economic Study. Front Cell Infect Microbiol. 2022;12:804611.

27. Soloaga R, Barcán L, Bettiol M, Carrión N, Cornistein W, Cristofano AD, et al. Estudio multicéntrico argentino sobre la utilidad del panel de neumonía de FilmArray®. Acta Bioquímica Clínica Latinoam. 2021;55(3):347–55.

28. Chen H, Weng H, Lin M, He P, Li Y, Xie Q, et al. The Clinical Significance of FilmArray Respiratory Panel in Diagnosing Community-Acquired Pneumonia. BioMed Res Int. 2017;2017:7320859.

29. Buchan B, Windham S, Balada-Llasat J, Leber A, Harrington A, Relich R, et al. Practical Comparison of the BioFire FilmArray Pneumonia Panel to Routine Diagnostic Methods and Potential Impact on Antimicrobial Stewardship in Adult Hospitalized Patients with Lower Respiratory Tract Infections. J Clin Microbiol. 2020 Jun 24;58(7):e00135-20.

30. Enne VI, Aydin A, Baldan R, Owen DR, Richardson H, Ricciardi F, et al. Multicentre evaluation of two multiplex PCR platforms for the rapid microbiological investigation of nosocomial pneumonia in UK ICUs: the INHALE WP1 study. Thorax. 2022 Jan 13;thoraxjnl-2021-216990.

31. Gadsby NJ, McHugh MP, Forbes C, MacKenzie L, Hamilton SKD, Griffith DM, et al. Comparison of Unyvero P55 Pneumonia Cartridge, in-house PCR and culture for the identification of respiratory pathogens and antibiotic resistance in bronchoalveolar lavage fluids in the critical care setting. Eur J Clin Microbiol Infect Dis Off Publ Eur Soc Clin Microbiol. 2019 Jun;38(6):1171–8.

32. Crémet L, Gaborit B, Bouras M, Drumel T, Guillotin F, Poulain C, et al. Evaluation of the FilmArray® Pneumonia Plus Panel for Rapid Diagnosis of Hospital-Acquired Pneumonia in Intensive Care Unit Patients. Front Microbiol. 2020;11:2080.

33. BIOFIRE® FILMARRAY® Pneumonia Panel plus. In: bioMérieux España [Internet]. Madrid: bioMérieux; [cited 2022 Oct 31]. Available from: https://www.biomerieux.es/diagnostico-clinico/productos/biofirer-filmarrayr-pneumonia-panel-plus

34. Lee SH, Ruan SY, Pan SC, Lee TF, Chien JY, Hsueh PR. Performance of a multiplex PCR pneumonia panel for the identification of respiratory pathogens and the main determinants of resistance from the lower respiratory tract specimens of adult patients in intensive care units. J Microbiol Immunol Infect. 2019 Dec;52(6):920–8.

35. Poole S, Tanner AR, Naidu VV, Borca F, Phan H, Saeed K, et al. Molecular point-of-care testing for lower respiratory tract pathogens improves safe antibiotic de-escalation in patients with pneumonia in the ICU: Results of a randomised controlled trial | Elsevier Enhanced Reader. J Infect. 2022;S063-4453(22):00533–3.

36. Green J, Clark A, Hayes T, Rasband K, Killpack J, Jones D, et al. Fully Automated Lysis and Purification of Multiple Gastrointestinal Pathogen Nucleic Acid for Simultaneous Detection from a Single Stool Sample. Salt Lake City: BioFire Diagnostics; 2014.

37. Corcoy M, Gallego JI. Consentimiento por representación. Barcelona: Fundació Víctor Grífols i Lucas; 2010.

A. | Annexes

Annex 1. **APACHE II** (Acute Physiologic and Chronic Health Evaluation)

APACHE II SCORE – Acute Physiologic and Chronic Health Evaluation									
(A) Acute Physiological Score									
	High abnormal range				Normal range	Low abnormal range			
APS	4	3	2	1	0	1	2	3	4
Rectal temperature (ºC)	≥41	39-40,9	-	38,5-38,9	36-38,4	34-35,9	32-33,9	30-31,9	≤29,0
Mean arterial pressure (mmHg)	≥169	130-159	110-129	-	70-109		50-69	-	<50
Heart rate-ventricular response (lpm)	≥180	140-179	110-129	-	70-109		55-69	40-54	<40
Respiratory rate (rpm)	≥50	35-49		25-34	12-24	10-11	6-9		<6
Oxygenation - FiO2 ≥0,5 (A-a DO_2) - FiO2 ≤0,5 (PaO_2)	499	350-499	200-349		>200 <70	61-70		56-70	<56
Arterial pH	>7,9	7,60-7,69		7,50-7,59	7,33-7,49		7,25-7,32	7,15-7,24	<7,15
Serum Na (mmol/l)	≥180	160-179	155-159	150-154	130-149		120-129	111-119	<111
Serum K (mmol/l)	>6,9	6,0-6,9		5,5-5,9	3,5-5,4	3,0-3,4	2,5-2,9		<2,5
Creatinine* (mg/dl)	>3,4	2,0-3,4	1,5-1,9		0,6-1,4		<0,6		
Haematocrit (%)	≥60		50-59,9	46-49,9	30-45,9		20-29,9		<20
White blood cell count (x1000/mm^3)	≥40		20-39,9	15-19,9	3-14,9		1-2,9		<1
TOTAL APS									

(B) 15 – Glasgow Coma Score	
15 - GCS	

(C) Age points	
Age (years)	Points
≤44	0
45-54	2
55-64	3
65-74	5
≥75	6

(D) Previous chronic disease	
Liver:	2
Cardiovascular	2
Respiratory: severe COPD, hypercapnia, home O_2, pulmonary hypertension	2
Immunocompromised	2
Renal: chronic dialysis	2

A Score for APS	**B** Score for GCS	**C** Score for age	**D** Score for previous chronic disease

TOTAL APACHE II SCORE (A + B + C + D)	

Annex 2. **SOFA Score** (Sequential Organ Failure Assessment)

SOFA SCORE – Sequential Organ Failure Assessment					
	0	1	2	3	4
Respiratory PaO_2/FiO_2 (mmHg)	>400	<400	<300	<200	<100
or SaO_2/FiO_2 [1]		221-301	142-220	67-141	<67
Coagulation Platelets $10^3/mm^3$	>150	<150	>100	>50	<20
Liver Bilirubin (mg/dl)	<1,2	1,2-1,9	2,0-5,9	6,0-11,9	>12
Cardiovascular Arterial pressure or [2] (dose in µg/kg/min)	MAP ≥70mmHg	MAP <70mmHg	Dopamine <5 or dobutamine at any dose	Dopamine at 5, 1-15 // Epinephrine ≤0,1 // Norepinephrine ≤0,1	Dopamine at >15 // Epinephrine >0,1 // Norepinephrine >0,1
Neurologic GCS score	15	13-14	10-12	6-9	<6
Renal Creatinin (mg/dl) or urinary flux (ml/d)	<1,2	1,2-1,9	2,0-3,4	3,5-4,9	>5,0
				<500	<200

*Abbreviations: PaO_2: Partial Pressure of Oxygen // FiO_2: Fraction of Inspired Oxygen // $SatO_2$: Oxygen Saturation // MAP: Mean arterial pressure
[1] PaO_2/FiO_2 is the preferred ratio, but if PaO2 is not available, $SatO_2/FiO_2$ ratio will be used
[2] Vasoactive drugs administered for at least 1h to maintain MAP above 65mmHg (doses are expressed in in µg/kg/min)

Annex 3. Murray and Washington's criteria

Murray and Washington's criteria to assess the quality of the respiratory sample

Murray and Washington's grading system to assess the quality of the respiratory sample evaluates the balance between upper tract epithelial cells (indicative of oropharyngeal contamination) and polymorphonuclear cells (indicative of an infectious process).

Grade	Number of squamous epithelial cells/low magnification field	Number of polymorphonuclear cells/low magnification field
1	>25	<10
2	>25	10-25
3	>25	>25
4	10-25	>25
5	**<10**	**>25**

For the samples to be valuable, they must be of good or optimal quality, which means that they must be representative of the lower airway → >25PMN and <10 epithelial cells/low magnification field.

This system was created to determine the quality of sputum samples and its use has subsequently been extrapolated to tracheal and bronchial aspirates, but it is not applicable to samples of alveolar origin (bronchoalveolar lavage, telescoping catheter, biopsies), nor it is applicable to cases of neutropenia, haemorrhage or samples lacking cells or with the presence of other type of cells (lymphocytes, globet).

Annex 4. Data collection form

Document de recollida de dades

Nom del projecte: Potential Impact of the FilmArray Multiplex PCR as compared to standard of care testing on Antimicrobial Therapy Guidance and Clinical Outcome for Ventilator-Associated Pneumonia in Critically Ill Patients		
Data de naixement	___ / ___ / _____	
Sexe	◯ Femení	◯ Masculí
Tècnica diagnòstica	◯ Cultiu convencional	◯ FilmArray + cultiu convencional
Puntuació APACHE II a entrada UCI		
Factors de risc per MDRO		
Agent etiològic identificat	◯ Agent identificat	◯ Agent no identificat
Modificació del tractament		
Sí / No	◯ Sí (tractament modificat)	◯ No (tractament no modificat)
Temps fins modificació	____ dies	
Causa de la modificació	◯ Escalada	◯ Desescalada
Efectes adversos de l'antibiòtic	◯ Sí (especificar causa)	◯ No
Durada del tractament		
Adquisició de MDRO	◯ Sí	◯ No
Positivitat d'altres cultius	◯ Sí (especificar quin)	◯ No
Puntuació SOFA	al diagnòstic de VAP	
	al cap de 7 dies	
Canvi SOFA	◯ Millor	◯ Igual o pitjor
Dies ventilació mecànica		
Dies d'estada a UCI		
Dies d'estada a l'hospital		
Mortalitat als 30 dies	◯ Defunció	◯ No defunció

Nom del responsable

Data: ___ / ___ / _____

Annex 5. Information sheet

Full d'informació al pacient

Nom del projecte: Potential Impact of the FilmArray Multiplex PCR as compared to standard of care testing on Antimicrobial Therapy Guidance and Clinical Outcome for Ventilator-Associated Pneumonia in Critically Ill Patients
Centre assistencial: Parc Hospitalari Martí i Julià – Hospital de Santa Caterina
Investigadors principals: Dra. Mireia Jurado Ramal i Dr. Josep Miquel Morales Pedrosa

Introducció

Benvolgut/da,

Ens dirigim a vostè per informar-lo sobre un estudi d'investigació en el qual se'l convida a participar, que es realitzarà a la Unitat de Cures Intensives de l'Hospital Santa Caterina. L'estudi ha estat aprovat pel Comitè d'Ètica i Investigació Clínica (CEIC) de Girona, d'acord amb la legislació vigent.

La finalitat d'aquest document és proporcionar-li tota la informació necessària sobre l'estudi per tal que conegui en què consistirà i pugui decidir si accepta o no accepta participar-hi. Li demanem que llegeixi el full informatiu amb atenció i que, en cas de qualsevol dubte, es posi en contacte amb els responsables per poder-lo solucionar.

Participació voluntària

La seva participació en aquest estudi és totalment voluntària. En cas que decideixi no participar, les seves dades no es tindran en compte en cap moment i no li suposarà cap perjudici en un futur. En qualsevol punt de l'estudi, vostè té el dret a revocar el seu consentiment, sense que això suposi un canvi en la seva atenció sanitària.

Objectiu de l'estudi

L'estudi té com a principal objectiu avaluar l'impacte d'una nova tècnica diagnòstica molecular (FilmArray) en guiar l'elecció del tractament antimicrobià i l'evolució clínica dels pacients.

Descripció de l'estudi

L'estudi inclourà un total de 200 pacients majors de 18 anys ingressats a la Unitat de Cures Intensives de l'Hospital Santa Caterina diagnosticats amb pneumònia associada a la ventilació als quals s'hagi de proporcionar tractament antimicrobià.

Per tal de poder avaluar els objectius desitjats, es dividiran els pacients en dos grups de forma totalment aleatòria, dels quals s'obtindran mostres respiratòries que s'enviaran a analitzar.

- En els pacients assignats al grup 1, s'analitzarà la mostra obtinguda amb la tècnica diagnòstica de rutina actual, el cultiu convencional.
- En els pacients assignats al grup 2, s'analitzarà la mostra obtinguda amb la tècnica diagnòstica de rutina actual, el cultiu convencional i, a més a més, amb la tècnica diagnòstica d'estudi, el FilmArray,

En ambdós casos, les mostres del pacient seran analitzades amb la prova diagnòstica de rutina que s'ha estat fent servir fins ara per diagnosticar els casos de pneumònia associada a la ventilació. El tractament dels pacients es farà segons els resultats de les proves diagnòstiques utilitzades, seguint les recomanacions de les guies de pràctica clínica més recents.

En qualsevol cas, la sospita clínica del metge responsable prevaldrà per sobre del que suggereixin els resultats obtinguts amb el FilmArray.

Riscs i beneficis

El FilmArray és un mètode diagnòstic de recent aparició que ha estat aprovat per la FDA (Food and Drug Administration, agència del Departament de Salut i Serveis Humans dels EEUU).

Tenint en compte que l'estudi es basa en canviar el mètode d'anàlisi microbiològic de les mostres respiratòries obtingudes, i no en el maneig terapèutic directe dels pacients, els riscs que pot comportar l'aplicació d'aquest nou mètode diagnòstic són els associats a l'ús dels fàrmacs antimicrobians ja comercialitzats i utilitzats de forma habitual en la pràctica clínica de l'hospital.

Confidencialitat i protecció de dades personals

La informació recollida en aquest estudi serà totalment confidencial, tot respectant la normativa de protecció de dades personals nacional *("Ley Orgánica 3/2018 de 5 de diciembre, de Protección de Datos Personales y garantia de los derechos digitales"*) i europea ("*Reglamento (UE) 2016/679 del Parlamento Europeo y del Consejo, del 27 de abril de 2016, relativo a la protección de las persones físicas en lo que repecta al tratamiento de datos personales y a la libre circulación de estos datos"*) i assegurant el seu compliment en tot moment. Les seves dades seran accessibles només pels membres de l'equip de recerca, els quals les emmagatzemaran en una base de dades anònima.

De la mateixa manera, en cas de publicació de resultats a través de publicacions o congressos, les seves dades seran tractades de forma anònima.

Contacte en cas de dubte

Si durant la seva participació té algun dubte o necessita obtenir més informació, pot posar-se en contacte amb els responsables principals de la investigació. Les dades de contacte se li proporcionaran en cas que, finalment, desitgi participar.

Universitat de Girona
Facultat de Medicina

Annex 5. Informed Consent Form

Document de Consentiment Informat

Nom del projecte: Potential Impact of the FilmArray Multiplex PCR as compared to standard of care testing on Antimicrobial Therapy Guidance and Clinical Outcome for Ventilator-Associated Pneumonia in Critically Ill Patients
Centre assistencial: Parc Hospitalari Martí i Julià – Hospital de Santa Caterina
Investigadors principals: Dra. Mireia Jurado Ramal i Dr. Josep Miquel Morales Pedrosa

Jo, _____ (Nom i Cognoms del participant), manifesto que:

◯ He llegit i entès el full informatiu que se m'ha entregat sobre l'estudi

◯ He tingut l'oportunitat de fer les preguntes pertinents

◯ He rebut respostes satisfactòries a les preguntes realitzades

◯ He rebut informació suficient respecte l'estudi

◯ Entenc que la meva participació és voluntària

◯ Comprenc que puc retirar-me de l'estudi en qualsevol moment, sense haver de donar explicacions i sense que això repercuteixi a la meva atenció sanitària.

◯ Estic d'acord en què el meu consentiment i les dades resultants de la meva participació en el projecte estiguin a disposició de l'equip d'investigació, sempre respectant la confidencialitat i amb la garantia que les dades no estaran disponibles públicament de manera que se'm pugui identificar.

Presto la meva conformitat per a participar en l'estudi, confirmo que he llegit el full d'informació i estic conforme amb el seu contingut. Rebo una còpia firmada i datada d'aquest full d'informació i consentiment informat per guardar-los i poder consultar-los en un futur.

Firma del participant	**Firma del responsable**
Data: ___ / ___ / _____	Data: ___ / ___ / _____

Annex 6. Informed Consent Form by representation

Document de Consentiment Informat per representació

Nom del projecte: Potential Impact of the FilmArray Multiplex PCR as compared to standard of care testing on Antimicrobial Therapy Guidance and Clinical Outcome for Ventilator-Associated Pneumonia in Critically Ill Patients
Centre assistencial: Parc Hospitalari Martí i Julià – Hospital de Santa Caterina
Investigadors principals: Dra. Mireia Jurado Ramal i Dr. Josep Miquel Morales Pedrosa

Jo, _____ (Nom i Cognoms del representant), representant de _____ (Nom i Cognoms del participant), manifesto que:

◯ Actuo en la seva representació per decidir en els seus interessos i manifestar la seva conformitat en participar en l'estudi confiant en que, si es trobés amb capacitat per decidir, ell/ella així ho voldria.

◯ He llegit i entès el full informatiu que se m'ha entregat sobre l'estudi

◯ He tingut l'oportunitat de fer les preguntes pertinents

◯ He rebut respostes satisfactòries a les preguntes realitzades

◯ He rebut informació suficient respecte l'estudi

◯ Entenc que la participació de _____ és voluntària i comprenc que pot retirar-se de l'estudi en qualsevol moment, sense haver de donar explicacions i sense que això repercuteixi en la seva atenció sanitària.

◯ Estic d'acord en què el meu consentiment i les dades resultants de la participació de _____ en el projecte estiguin a disposició de l'equip d'investigació, sempre respectant la confidencialitat i amb la garantia que les dades no estaran disponibles públicament de manera que se'l pugui identificar.

Presto la meva conformitat per a que _____ participi en l'estudi, confirmo que he llegit el full d'informació i estic conforme amb el seu contingut. Rebo una còpia firmada i datada d'aquest full d'informació i consentiment informat per guardar-los i poder consultar-los en un futur.

Firma del participant	**Firma del responsable**
Data: ___ / ___ / _____	Data: ___ / ___ / _____

Annex 7. Withdrawal of Informed Consent

Document de Revocació del Consentiment Informat

Nom del projecte: Potential Impact of the FilmArray Multiplex PCR as compared to standard of care testing on Antimicrobial Therapy Guidance and Clinical Outcome for Ventilator-Associated Pneumonia in Critically Ill Patients
Centre assistencial: Parc Hospitalari Martí i Julià – Hospital de Santa Caterina
Investigadors principals: Dra. Mireia Jurado Ramal i Dr. Josep Miquel Morales Pedrosa

Jo, _____ (Nom i Cognoms del participant) manifesto que, un cop coneguda la meva inclusió en l'estudi:

◯ He llegit i entès el full informatiu que se m'ha entregat sobre l'estudi

◯ He tingut l'oportunitat de fer les preguntes pertinents

◯ He rebut respostes satisfactòries a les preguntes realitzades

◯ He rebut informació suficient respecte l'estudi

◯ Entenc que la meva participació és voluntària

Revoco el consentiment prestat per _____ amb data ___ / ___ / _____ i ja no desitjo prosseguir amb l'estudi *Potential Impact of the FilmArray Multiplex PCR as compared to standard of care testing on Antimicrobial Therapy Guidance and Clinical Outcome for Ventilator-Associated Pneumonia in Critically Ill Patients,* sabent que això no repercutirà en la seva atenció sanitària.

.

Firma del participant	**Firma del responsable**
Data: ___ / ___ / _____	Data: ___ / ___ / _____