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# IMPLEMENTATION OF THE FILMARRAY PNEUMONIA PANEL IN PATIENTS WITH NOSOCOMIAL PNEUMONIA

A QUASI-EXPERIMENTAL STUDY

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**FINAL DEGREE PROJECT**

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*En primer lloc, al Dr. Albert Gómez, gràcies per tots els consells que m'has donat, per guiar-me les pràctiques i el treball, i sobretot per ensenyar-me la part més humana i bonica de la medicina.*

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*A la Dra. Maite Serrando, per ser el meu àngel de la guarda al llarg de la carrera.*

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*Aquest treball també és vostre.*

*Finalment, a tu iaia,  
T'estimo i et trobo a faltar.*

## ABBREVIATIONS:

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- **ARDS:** Acute Respiratory Distress Syndrome
- **BAS:** Bronchial Aspirate
- **BAL:** Bronchoalveolar Lavage
- **BMI:** Body Mass Index
- **CAP:** Community-Acquired Pneumonia
- **CEIC:** Comitè d'Ètica d'Investigació Clínica
- **CFU:** Colony Forming Unite
- **CI:** Co-Investigator
- **CKD:** Chronic Kidney Disease
- **CMV:** Cytomegalovirus
- **CNS:** Central Nervous System
- **CO:** Collaborators
- **COPD:** Chronic Obstructive Pulmonary Disease
- **CPE:** Carbapenemase-Producing *Enterobacteriaceae*
- **CT:** Computed Tomography
- **CXR:** Chest X-Ray
- **DNA:** Deoxyribonucleic Acid
- **ESBL:** Extended-Spectrum Beta-lactamase
- **FDA:** Food and Drug Administration
- **HBP:** High Blood Pressure
- **ICU:** Intensive Care Unite
- **IDIBGI:** Institut d'Investigació Biomèdica de Girona
- **HAP:** Hospital-Acquired Pneumonia
- **HCAP:** Health Care-Associated Pneumonia
- **HSV:** Herpes Simplex Virus
- **LTE:** Limitation of Therapeutic Effort
- **MDR:** Multidrug-Resistant
- **MDRO:** Multidrug-Resistant Organisms
- **MI:** Main investigator
- **MRSA:** Methicillin-Resistant *Staphylococcus aureus*
- **MSSA:** Methicillin-Sensitive *Staphylococcus aureus*

- **NP:** Nosocomial Pneumonia
- **PC:** Project Coordinator
- **PCR:** Polymerase Chain Reaction
- **PDR:** Pandrug-Resistant
- **PPI:** Proton-Pump Inhibitor
- **PS:** Professional Statistician
- **PSB:** Protected Specimen Brushing
- **RT:** Research Team
- **RNA:** Ribonucleic Acid
- **RSV:** Respiratory Syncytial Virus
- **SEMI:** Sociedad Española de Medicina Interna
- **VAP:** Ventilator-Associated Pneumonia
- **XDR:** Extensively Drug-Resistant
- **XDRO:** Extensively Drug-Resistant Organism
- **WHO:** World Health Organization

# INDEX:

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|   |           |
|---|-----------|
| <b>1. ABSTRACT.....</b>                                 | <b>7</b>  |
| <b>2. INTRODUCTION.....</b>                             | <b>8</b>  |
| <b>2.1. OVERVIEW OF PNEUMONIA.....</b>                  | <b>8</b>  |
| 2.1.2 Epidemiology .....                                | 9         |
| 2.1.3 Aetiology .....                                   | 10        |
| 2.1.4 Pathophysiology .....                             | 15        |
| 2.1.5 Risk Factors.....                                 | 16        |
| 2.1.6 Diagnosis.....                                    | 18        |
| 2.1.7 Therapeutic management .....                      | 25        |
| 2.1.8 Prevention .....                                  | 29        |
| <b>2.2 The development of Molecular Techniques.....</b> | <b>31</b> |
| 2.2.1 Film Array .....                                  | 31        |
| 2.2.2 FilmArray Pneumonia <i>Plus</i> Panel .....       | 32        |
| <b>2.3 Antimicrobial Resistance .....</b>               | <b>36</b> |
| 2.3.1 Definition.....                                   | 36        |
| 2.3.2 Mechanisms antibiotic resistance .....            | 37        |
| <b>3. JUSTIFICATION.....</b>                            | <b>41</b> |
| <b>4. HYPOTHESES.....</b>                               | <b>43</b> |
| 4.1 Main hypothesis:.....                               | 43        |
| 4.2 Secondary hypothesis. ....                          | 43        |
| <b>5. OBJECTIVES.....</b>                               | <b>43</b> |
| 5.1 Main objective.....                                 | 43        |
| 5.2 Secondary objectives .....                          | 43        |
| <b>6. MATERIAL AND METHODS .....</b>                    | <b>44</b> |
| <b>6.1 Study design.....</b>                            | <b>44</b> |
| 6.2.1 Inclusion criteria.....                           | 44        |
| 6.2.2 Exclusion criteria .....                          | 45        |
| 6.2.3 Withdrawal criteria .....                         | 45        |
| <b>6.3 Sample .....</b>                                 | <b>46</b> |
| 6.3.1 Sample size .....                                 | 46        |
| 6.3.2 Sample selection .....                            | 46        |
| 6.3.3 Study duration .....                              | 46        |
| <b>6.4 Variables and measurements .....</b>             | <b>47</b> |

|   |           |
|---|-----------|
| 6.5 Study intervention.....   | 51        |
| 6.6 Safety.....   | 52        |
| 6.7 Data collection.....  | 53        |
| 6.8 Follow-up.....  | 53        |
| <b>7. STATISTICAL ANALYSIS.....</b>                                   | <b>54</b> |
| 7.1 Descriptive analysis.....   | 54        |
| 7.2 Bivariate inference.....  | 54        |
| 7.3 Multivariate analysis.....  | 54        |
| <b>8. ETHICAL AND LEGAL CONSIDERATIONS.....</b>                       | <b>55</b> |
| <b>9. WORKING PLAN AND CHRONOGRAM.....</b>                            | <b>58</b> |
| 9.1 Research team members.....  | 58        |
| 9.2 Work plan.....  | 59        |
| 9.3 Chronogram.....   | 61        |
| <b>10. BUDGET.....</b>  | <b>62</b> |
| 10.1 Non-included expenses.....                                       | 62        |
| 10.2 Costs included.....  | 63        |
| <b>11. LIMITATIONS AND STRENGTHS.....</b>                             | <b>65</b> |
| <b>12. CLINICAL AND HEALTHCARE IMPACT.....</b>                        | <b>67</b> |
| <b>13. FEASIBILITY.....</b>   | <b>68</b> |
| <b>14. BIBLIOGRAPHY.....</b>  | <b>69</b> |
| <b>15. ANNEXES.....</b>   | <b>75</b> |
| - <b>ANNEX 1:</b> Murray-Washington's criteria.....                   | 75        |
| - <b>ANNEX 2:</b> Data collection from Santa Caterina Laboratory..... | 76        |
| - <b>ANNEX 3:</b> Case report form.....                               | 78        |
| - <b>ANNEX 4:</b> Informative Document.....                           | 79        |
| - <b>ANNEX 5:</b> Informed Consent Form.....                          | 82        |
| - <b>ANNEX 6:</b> Withdrawal of Informed Consent.....                 | 83        |

## INDEX OF TABLES:

---

|   |    |
|---|----|
| - <b>Table 1:</b> Aetiology according to time of acquisition and associated risk of MDRO... | 11 |
| - <b>Table 2:</b> Risk factors for nosocomial pneumonia.....                                | 16 |
| - <b>Table 3:</b> Risk factors for HAP caused by MDR or XDR microorganism.....              | 17 |
| - <b>Table 4:</b> Comparison of non-invasive and invasive diagnostic techniques.....        | 23 |
| - <b>Table 5:</b> Causes of lack of response to empirical treatment.....                    | 27 |
| - <b>Table 6:</b> BioFire FilmArray Pneumonia Plus Panel targets .....                      | 32 |
| - <b>Table 7:</b> Description of the variables included in the study .....                  | 50 |
| - <b>Table 8:</b> Chronogram of the study work plan.....                                    | 61 |
| - <b>Table 9:</b> Total cost of the study .....   | 64 |

## 1. ABSTRACT

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**BACKGROUND:** Hospital-acquired pneumonia is a frequent nosocomial infection in patients admitted to internal medicine wards and a major cause of antibiotic overuse. Standard culture methods currently take at least five days to identify pathogens and their antibiotic susceptibility, leading to broad-spectrum empirical treatment. This widespread practice increases the risk of acquiring multi-drug resistant organisms, thereby exacerbating antibiotic resistance. New molecular diagnostic methods, such as FilmArray rapid multiplex PCR, offer an alternative to reduce pathogen identification time to less than one hour, as well as associated multiresistance, potentially improving patient outcomes and reducing the development of multidrug-resistant organisms, thus addressing the global challenge of antibiotic resistance.

**OBJECTIVES:** To assess whether the implementation of molecular testing using FilmArray and subsequent targeted treatment of nosocomial pneumonias, as opposed to conventional culture and empirical treatment, will improve long-term hospital antimicrobial resistance and clinical patient outcomes, reducing associated complications, hospital stays, readmissions and mortality rates.

**STUDY PARTICIPANTS:** This study includes adult patients ( $\geq 18$  years) admitted to Santa Caterina Hospital, diagnosed with hospital-acquired pneumonia, and able to provide a sufficient volume of sputum sample obtained to perform FilmArray.

**DESIGN AND METHODS:** This is a prospective, single-centre, quasi-experimental study. The study will consist of a before and after evaluation, where in the first phase (pre-intervention) 37 participants will be enrolled, diagnosed using conventional culture and empirically treated. In the second phase of the study (post-intervention), 111 patients will be recruited and the FilmArray Pneumonia Panel will be implemented. These patients will receive treatment based on the results obtained from the technique. The aim is to determine if this intervention can reduce antibiotic resistance at Santa Caterina Hospital. The study will last for a total of 5 years.

**KEYWORDS:** *Hospital-acquired pneumonia, nosocomial pneumonia, molecular testing, FilmArray, multiplex PCR, antimicrobial resistance, antimicrobial stewardship, multidrug-resistant organisms.*



## 2. INTRODUCTION

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### 2.1. OVERVIEW OF PNEUMONIA

Pneumonia is defined as an acute infection characterised by inflammation and damage to the lung parenchyma, accompanied by the development of a new or progressive infiltrate and clinical findings suggestive of infection, such as new-onset of fever, purulent sputum, leukocytosis, and decreased oxygenation. Also, it may be accompanied by pleuritic pain, dyspnoea, chills and extrapulmonary symptoms.

It can be caused by a variety of microorganisms, including bacteria, viruses, and fungi (1), and is a major public health concern and one of the leading causes of morbidity and mortality worldwide.

Pneumonia can be classified, depending on where the infection occurred: (2)

- **Community-acquired pneumonia (CAP):** an infection acquired outside the hospital setting, affecting the general population or patients who have been hospitalised for less than 48 hours.
- **Hospital-acquired pneumonia (HAP) or Nosocomial Pneumonia (NP):** infection acquired by pathogens that are present in hospital settings, in patients after at least 48 hours of hospitalisation for another illness or condition.
  - o **Ventilator-associated pneumonia (VAP):** a subcategory of HAP occurring in patients after receiving mechanical ventilation. It represents more than 80% of pneumonia cases acquired in Intensive Care Unit (ICU) (3). It is characterised by distinct pathophysiology, microbiology and therapeutic considerations.
- **Health care-associated pneumonia (HCAP):** an infection acquired in lower-acuity healthcare settings, such as nursing homes and dialysis centres or through regular contact with healthcare facilities. According to recent guidelines (3,4), patients with this pneumonia are not at an increased risk of multidrug-resistant organisms (MDRO). For this reason, they do not recommend to use this term and this is the reason why it will not be used in this project.

### 2.1.1 Hospital-Acquired Pneumonia (HAP)

**Hospital-acquired pneumonia**, also referred to as **Nosocomial Pneumonia**, is an infection characterized by an inflammatory lung process that is absent at the time of hospital admission and develops more than 48 hours later (3).

The term '*early-onset*' has been used to refer to healthcare-associated infections that occur within the first 96 hours of hospital stay and it is typically caused by community-acquired microorganisms (*Pneumococcus* and *H. influenzae*), while '*late-onset*' is used for those infections that occur thereafter and they are usually caused by hospital-acquired microorganisms (5).

This type of pneumonia holds significant clinical importance due to its heightened morbidity and mortality, particularly in cases caused by MDRO. Moreover, the compromised quality of life, prolonged hospital stays and considerable consumption of healthcare resources are also affected.

HAP and VAP present similar microbiology and pathophysiology. However, it is quite different when considering associated mortality. Therefore, it is necessary to make distinctions among: (3)

- HAP not requiring mechanical ventilation.
- HAP requiring mechanical ventilation.
- HAP acquired during mechanical ventilation, also known as VAP.

### 2.1.2 Epidemiology

Nowadays, pneumonia still remains one of the leading causes of hospitalisation and mortality worldwide. According to the World Health Organisation (WHO), in 2019, lower respiratory tract infections remained the world's most deadly communicable disease, ranked as the fourth leading cause of death globally, claiming 2,6 million lives (6).

Despite advances in the understanding of contributing causes and prevention, nosocomial pneumonia continues to be frequent complications of hospital care. HAP is the second leading cause of nosocomial infection, occurring at a rate of 5 to more than 20 cases per 1.000 hospital admissions, increasing hospital stay by an average of 7 to 9 days per patient and representing an extra cost of hospital expensed (1).

In another study in 2012 in the United States involving a sample of over 7 million hospitalised patients, the observed incidence rate of HAP was 3.63 cases per 1,000 patient-days (7).

These infections have a negative impact on patient outcomes. Although HAP is traditionally perceived as less severe than VAP, approximately 50% of those patients have several complications, including respiratory or renal failure, septic shock and empyema. This particularly occurs in patients who develop HAP in ICU, where the mortality rate approaches that of patients with VAP (8). Numerous studies have also shown that HAP increases mortality on its own, increasing the risk of death by about 8.4 times (9).

### 2.1.3 Aetiology

Nosocomial pneumonia can be caused by a wide range of pathogens. Studies have shown that there are no differences between HAP and VAP (10). Bacterial infections are the cause of most infections, with *Pseudomonas aeruginosa* and *Staphylococcus aureus* being the most isolated pathogens. Polymicrobial etiology can also be found. Fungi and viruses typical affect immunocompromised patients (11).

During the anamnesis, it is necessary to consider apart from medical history, toxic habits, contact with animals, occupation, sexual and travel history because they can help to guide the aetiological diagnostic approach. In addition, one of the most important things is to identify risk factors associated with the probability of acquiring HAP from opportunistic and MDRO because it has implications for treatment and prognosis.

Traditionally, based on the 2005 ATS/ISDA guidelines (1), it has been recommended to differentiate between *early-onset* (< 5 days) and *late-onset* (>5 days) pneumonia with the aim of tailoring treatment to the most probably aetiology. The first group was defined with low risk of MDRO due to antibiotic-sensitive bacteria and, therefore, with better prognosis. The second group is more associated with poor prognosis and increased mortality and morbidity.

**Table 1:** Aetiology according to time of acquisition and associated risk of MDRO

| Early-onset HAP<br>without MDRO risk factors:  | Late-onset HAP<br>with MDRO risk factors:                    |
|--|--|
| <i>Streptococcus pneumoniae</i>  | <i>Pseudomonas aeruginosa</i>                                |
| <i>Haemophilus influenzae</i>  | <i>Klebsiella pneumoniae</i> (ESBL +)                        |
| Methicillin-sensitive<br><i>Staphylococcus aureus</i> (MSSA)   | Methicillin-resistant<br><i>Staphylococcus aureus</i> (MRSA) |
| Gram-negative enteric bacilli:<br>- <i>Escherichia coli</i><br>- <i>Klebsiella pneumoniae</i><br>- <i>Enterobacter spp</i><br>- <i>Proteus spp</i><br>- <i>Serratia marcescens</i> | <i>Acinetobacter spp</i>                                     |
|  | <i>Legionella pneumophila</i>                                |
|  | Other non-fermentative<br>Gram-Negative bacteria             |

Some studies (12) have questioned whether there are specific differences in the microorganisms isolated from early and late samples. However, the latest ATS/ISDA guidelines in 2016 (4) concluded that the evidence reviewed suggests that, overall, patients who develop HAP after >5 days of hospitalisation have a higher risk of infection with MDRO than patients who develop HAP earlier in their hospital stay.

The microbiological pattern of nosocomial pneumonia varies according to geographical areas, hospitals, and the specific units in which patients are admitted. Therefore, the latest guidelines (4,13) recommend that the microbiological profile of nosocomial infections should be determined in every unit and hospital to increase the proportion of appropriate empirical treatment.

### **Bacterial infections**

The etiology of bacterial nosocomial pneumonias is primarily attributed to Gram-Negative bacteria, including *Pseudomonas aeruginosa*, *K. pneumoniae*, *E.Coli*, *Enterobacter spp.* and *Acinetobacter spp.*, followed by Gram-positive cocci (*Methicillin-resistant Staphylococcus aureus* [MRSA] or *Methicillin-sensitive Staphylococcus aureus* [MSSA]) (3,14).

**GRAM NEGATIVE BACTERIA:**

***Pseudomonas aeruginosa*** is a gram-negative opportunistic pathogen that is a leading cause of nosocomial infections and is responsible for 10% of all hospital-acquired infections, particularly pneumonia. It has been reported as the causative agent of CAP and HAP, often associated with ventilator use. *P. aeruginosa* is susceptible to a limited number of antibiotics (antipseudomonal penicillin and cephalosporins, carbapenems, and fluoroquinolones), and multi-drug resistant (MDR) *P. aeruginosa* infections are becoming an increasing problem in hospitals (15).

***Klebsiella pneumoniae*** is a gram-negative rod-shaped bacterium found as part of the normal flora of the human mouth and skin. However, when it is aspirated into the lungs it can cause alveolar damage leading to pneumonia. *Klebsiella* is an opportunistic pathogen accounting for 7-14% of HAP and is associated often with nosocomial infections in the elderly or immunocompromised patients. The mortality rate associated with *K. pneumoniae* infection is in part due to the emergence of antibiotic resistance genes (e.g. carbapenemases) in these bacteria (16).

***Escherichia coli*** is a gram-negative bacterium that is part of the normal flora of the intestines of humans. It is found in approximately 6-9% of CAP and HAP and is responsible for 1.2% of all pneumonia diagnoses in the United States. This bacterium acts as an opportunistic causal agent of pneumonia and the prognosis associated with *E. coli*-caused pneumonia is poorer than that for pneumonia caused by other bacteria and viruses. As with other *Enterobacteriaceae*, extended spectrum  $\beta$ -lactamases (ESBL) pose a significant antibiotic resistance problem (17).

***Acinetobacter baumannii*** is a non-fermentative, gram-negative coccobacillus that primarily acts as an opportunistic pathogen infecting critically ill patients. It is an uncommon member of the normal skin flora. HAP is the most common infection caused by *A. baumannii* although other nosocomial infections caused by *A. baumannii* are increasing in frequency. Multi-drug resistant strains demonstrate resistance to most antibiotic classes, including carbapenems (18).

**GRAM POSITIVE BACTERIA:**

***Staphylococcus aureus*** is a gram-positive coccus that grows in grape-like clusters. A common, opportunistic bacterium, *S. Aureus* is capable of causing a wide range of diseases and is considered the most clinically important human pathogen in the *Staphylococcus* genus. It is among the most common etiologic agents in lower respiratory tract infections, being at 17% the most frequently reported isolate in HAP.

This bacterium possesses extensive virulence factors, has various strategies to evade the host immune response and has become resistant to many therapeutic agents. It is estimated that approximately 40% of *S. aureus* isolates may be methicillin resistant. The primary mediator of methicillin resistance in staphylococci is acquisition of the *mecA* gene (19–21).

**Viral infections**

As mentioned above, the incidence of HAP caused by viruses is infrequent in immunocompetent patients. Virus-induced pneumonia may arise from viral infection, bacterial superinfection or both circumstances. The most typical causes of viral HAP infections are due to *Adenovirus*, *Influenza A and B*, *Parainfluenza*, and *Respiratory Syncytial Virus (RSV)*. These infections are seasonal, mainly occurring during the colder months, particularly during the flu season.

*Influenza Virus A* is probably the most common viral cause of HAP, followed by *RSV*, more typical in children and immunocompromised patients. The *Adenovirus* and *Parainfluenza Virus* are not as common as *Influenza* and *RSV*, but they can still induce nosocomial pneumonia in certain cases. It is crucial to acknowledge that the prevalence of these viruses can fluctuate depending on the region, season, and specific characteristics of patients as well as the hospital environment (1).

The incidence of *Herpes Simplex Virus (HSV)* and *Cytomegalovirus (CMV)* is notably reduced and mostly associated with patients with acute respiratory distress syndrome (ARDS) and those patients with corticosteroid therapy. These viruses are also frequently observed in patients admitted to the ICU who have had prolonged intubation, especially when bacterial agents have not been detected (22).

Finally, it is essential to consider the SARS-CoV-2 pandemic. Although there is limited knowledge about hospital-acquired infections in patients with SARS-CoV-2, there is evidence that some patients experience ARDS, systemic inflammation, and a prolonged disease course necessitating sustained mechanical ventilation.

Thus, COVID-19 patients themselves face a substantial risk of developing secondary infections. The most common bacterial pathogens are *Klebsiella pneumoniae*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. The incidence of bacterial superinfection in nosocomial pneumonia in patients with COVID-19 varies depending on the studied population, but it is estimated to be between 10-20%. The patients that have more risk are the ones with mechanical ventilation. The incidence of VAP due to SARS-CoV-2 infection is higher (33%) in comparison with the general ICU population (10%) (23).

Consequently, this pathogen should be taken into account when suspecting nosocomial pneumonia of viral origin, as it further complicates the already challenging clinical management of the patients (24,25).

### **Fungal infections**

Regarding nosocomial pneumonias caused by fungi, these are only found in immunocompromised patients, organ transplant recipients, or neutropenic patients, and are very rare in immunocompetent individuals. The main etiological agents are *Aspergillus fumigatus* and *Candida spp.*

- Infections caused by *Aspergillus* species indicate a potential spore-borne transmission and may be linked to an environmental source, such as contaminated air ducts or hospital construction sites.
- The identification of *Candida spp.* in respiratory samples mostly suggests colonization in the lower respiratory tract rather than being the real cause of HAP and VAP. It is typical of ICU intubated patients and rarely requires antifungal therapy. Nevertheless, colonization by *Candida* contribute to the development of VAP by *P. aeruginosa* and other MDRO, exacerbating the prognosis (1,22).

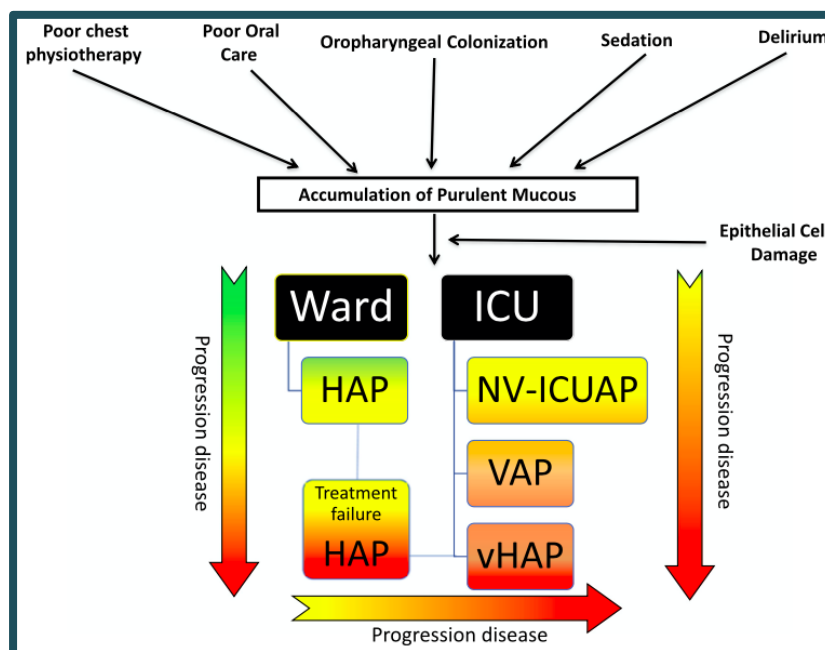
### 2.1.4 Pathophysiology

The pathophysiology of nosocomial pneumonia is multifactorial although the most frequent mechanism entails the aspiration of microorganisms colonising the oropharynx or gastrointestinal tract (1).

Aspiration happens in up to 45% of healthy individuals while they are sleeping, but it has no negative effects due to oropharyngeal microbiota containing commensal microorganism. However, in hospitalised patients the combination of depressed immune system, suppression of swallowing and cough reflex, the weakened clearance of the mucociliary system in the respiratory tract as well as the presence of comorbidities, malnutrition, and pathogenic organisms, make aspiration a significant contributing factor to nosocomial pneumonia (26).

Other factors, such as inadequate chest physiotherapy, suboptimal oral care, sedation or delirium, can also impact the accumulation of purulent mucus, thus, contributing to hospital-acquired pneumonia.

The agents responsible for colonisation and subsequent infection can be, exogenous (coming from the patient's environment), such as the inhalation of contaminated aerosols or nebulisers, contaminated endotracheal tubes or attire and hands of healthcare workers; on the other hand, they can be endogenous, originating from the patient's own bacterial microbiota or that which has been replaced by hospital microorganism (5,27).



**Figure 1:** Physiopathological approach of progression of nosocomial pneumonia from wards to ICU. Extracted from (19)



### 2.1.5 Risk Factors

When patients are hospitalised, several situations need to be considered for patient management, as they may be a risk factor for acquiring a nosocomial infection, facilitate silent aspiration of secretions, increase the quantity and pathogenicity of inoculated microorganisms and reduce local respiratory tract defences and systemic immunity.

These situations are often associated with changes in the host's defence mechanism (intrinsic risk factor) and/or diagnostic-therapeutic manipulations (extrinsic risk factor).

**Table 2:** Risk factors for nosocomial pneumonia. Extracted from (1,3,28)

| INTRINSEC RISK FACTORS<br>(Patient-related)   | EXTRINSEC RISK FACTORS<br>(Treatment-related)   |
|---|---|
| Advanced age, toxic habits (alcoholism or smoking) or malnutrition  | Prolonged hospital stay   |
| Family member cohabiting with a multidrug-resistant pathogen  | Prolonged or inappropriate antibiotic therapy   |
| Abnormal oropharyngeal or gastric colonization  | Artificial airways: endotracheal tubes, tracheostomy, enteral nutrition or nasogastric tube   |
| Underlying chronic disease: Chronic Obstructive Pulmonary Disease or other pre-existing lung disease, neuromuscular and Central Nervous System disease, diabetes mellitus, hematologic neoplasia, chronic renal failure or dialysis | Treatment with: <ul style="list-style-type: none"> <li>- Gastric antisecretory agents (H<sub>2</sub> receptor blockers or proton-pump inhibitors)</li> <li>- Cytotoxic agents</li> <li>- Central nervous system sedatives</li> <li>- Corticosteroids</li> </ul> |
| Altered state of consciousness or coma  | Aspiration or supine body position  |
| Traumatic brain injury  | Major or complicated surgery  |
| Sinusitis   | Transfusion > 4 U of hemoderivatives  |
| Multiple organ system failure and immunosuppression   | Aerosols sprays   |
| Other: acute respiratory distress syndrome (ARDS), acidosis, neutropenia, hypotension and shock.  | Poor in-hospital control of infections: lack of hand washing, no replacement of gloves, poor isolation of the patient   |

In addition, recent guidelines (4,13) highlight that to select the appropriate empirical antibiotic treatment, apart from considering the severity of pneumonia, it must be considered the presence or absence of clinical risk factor for infections by multidrug-resistant (MDR) or extensively drug-resistance (XDR) organism in the specific hospital setting.

**Table 3:** Risk factors for HAP caused by MDR or XDR microorganism according to clinical practice guidelines. Extracted from (3)

| Guideline for<br><i>ERS/ESICM/ESCMID/ALAT (13)</i>  | Guideline for<br><i>IDSA/ATS (4)</i>                  |
|---|---|
| Septic shock  | Septic Shock  |
| Previous antibiotic administration  | Antibiotic administration in the last 90 days or more |
| Hospital stay $\geq$ 5 days   | Acute Respiratory Distress Syndrome                   |
| Prevalence $\geq$ 25% of MDR pathogens in the hospital unit   | Acute kidney failure with replacement therapy         |
| Previous colonisation by MDR pathogens  | Immunosuppressive disease or therapy                  |
| <p><b>ABBREVIATIONS:</b><br/> ALAT: <i>Asociación Latinoamericana de Tórax</i>; ATS: <i>American Thoracic Society</i>; ERS: <i>European Respiratory Society</i>; ESCMID: <i>European Society of Clinical Microbiology and Infectious Diseases</i>; ESICM: <i>European Society of Intensive Care Medicine</i>; IDSA: <i>Infectious Diseases Society of America</i>; MDR: <i>Multidrug-Resistant</i>.</p> |   |

However, using clinical criteria as a means of addressing the initial treatment of infections can lead to an excess of unnecessary antibiotic treatment and in some cases, inadequate coverage. Additionally, it is challenging to identify highly specific risk factors for microorganisms with specific resistance patterns.

Furthermore, these factors are highly prevalent in patients admitted to the ICU, but there is no validation of these risk factors in non-ICU admitted patients. This becomes a limitation in validating these risk factors for all nosocomial pneumonias.

### 2.1.6 Diagnosis

The diagnosis of HAP can be challenging due to limited diagnostic tests and a wide differential diagnosis. According to recent guidelines (4), the diagnosis of HAP is mainly based on three fundamental aspects: clinical signs and symptoms, laboratory tests along with radiological evidence of new or progressive pulmonary infiltrates on Chest X-Ray (CXR) with the support of positive microbiological cultures from the lower respiratory tract.

#### **CLINICAL AND ANALYTICAL DIAGNOSIS**

The initial step in diagnosing pneumonia is to identify a decrease in oxygenation or an increase in oxygen requirements or need for respiratory support. This should be followed by new onset fever, leukocytosis ( $>12,000/\text{mm}^3$ ) or leukopenia ( $<4,000/\text{mm}^3$ ), purulent sputum, productive cough, or dyspnea among other findings.

Therefore, it is necessary for all patients to provide a complete medical history and undergo physical examination to determine the severity, exclude other potential sources of infection, and reveal the existence of specific conditions that may aid in identifying the aetiology of the pneumonia.

All patients should ask to have their vital signs measured: blood pressure, heart and respiratory rate, axillary temperature and pulse oximetry. A general blood test should be done with hemogram, coagulation and biochemistry including renal function and ions. Blood gas analysis is requested if the patient's baseline saturation is below 92%, if there is resting tachypnea or if the patient has multilobar pneumonia.

Biomarkers, including C-reactive protein and procalcitonin, have been suggested as additional diagnostic tools for HAP. However, at present they are not recommended (5,13) due to their lack of accuracy and restricted use in guiding the clinical course of patients. It is important to note that they should not be used to diagnose the patient or make therapeutic decisions regarding antimicrobial stewardship. In the 2020 Spanish update (29), it is determined that there is an exception with the measurement of serum procalcitonin because it can be useful when a treatment duration of more than 8 days is necessary, either due to an inadequate or difficult-to-assess clinical response, the emergence of pneumonia complications (abscess or pleural effusion), or in patients with infections caused by MDR pathogens.

Other biomarkers, alone or in combination, such as IL-6, the histidine-rich glycoprotein, anticitrullinated alpha-enolase peptide 1 and the neutrophil gelatinase-associated lipocalin have presented promising findings (30). Nevertheless, larger and better-designed studies are required to establish a potential role in the diagnosis of nosocomial pneumonia.

### **IMAGING DIAGNOSIS**

As mentioned previously, the diagnosis of HAP relies on the integration of clinical and radiological data, including the presence of new or progressing infiltrates that persist, typically manifested as bilateral pulmonary foci. The absence of such infiltrates markedly diminishes the likelihood of HAP, prompting the clinician to consider alternative diagnoses for the decline in respiratory function.

#### *Chest Radiography:*

Chest radiography is the most commonly used imaging technique for the diagnosis of nosocomial pneumonia because it is fast, readily available, non-expensive and minimally invasive. The low radiation exposure turns it into the preferred imaging technique. However, the test has low sensitivity (88.9%) and specificity (26.1%) when compared to the gold standard of histopathology lung biopsy for diagnose nosocomial pneumonia, particularly when using anteroposterior portable CXR in bedridden patients.

When the presence of infiltrates is combined with one clinical finding, the sensitivity ranges from 64.8% to 84.6%, and the specificity ranges from 33.3% to 36%. The rate improves to 91% when combined with three clinical findings (31).

It is important to note that non-infectious disorders, such as atelectasis, pleural effusion, chemical pneumonitis, asymmetric cardiogenic pulmonary edema, asymmetric noncardiogenic pulmonary embolism, pulmonary haemorrhage or contusion as well as cryptogenic organising pneumonia, can cause asymmetric pulmonary infiltrates too, being the main differential diagnosis.

#### *Low radiation computed tomography:*

Computed tomography (CT) imaging has demonstrated to be more sensitive than chest radiography in detecting non-specific radiographic findings and assessing potential lung complications (32).

Although CT scan is better than CXR, particularly in identifying hidden infiltrates in patients with “normal” CXR but clinical suspicion of pneumonia, the findings are often non-specific and frequently cannot be distinguished among other medical conditions. Concerns related to radiation exposure, cost, availability, and logistical challenges have restricted its application in pneumonia diagnosis up to the present time.

*Lung ultrasound:*

Lung ultrasound has recently been proposed as a promising diagnostic tool for nosocomial pneumonia, particularly for VAP, due to its potentially higher sensitivity than CXR. (33). The most useful sonographic signs include the appearance of subpleural consolidations in the anterior lung areas and lobar or sublobar consolidations with dynamic air bronchograms. It is important to always rely on clinical evidence or suspicion in conjunction with these signs (34).

Whilst ultrasound may prove to be a valuable tool in aiding diagnosis in the years to come, further studies are required to definitively determine its role in diagnosing nosocomial pneumonia.

**MICROBIOLOGICAL DIAGNOSIS**

When nosocomial pneumonia is suspected, it is essential to complement clinical evaluations with a microbiological sample, which is the second step in the diagnostic workup to identify the etiological pathogen causing the pneumonia (in HAP the etiological diagnosis is obtained in 40% and in VAP in 30%). In hospital-acquired pneumonia, unlike community-acquired pneumonia, achieving an etiological diagnosis is crucial to guide treatment as precisely as possible, with the aim of reducing the emergence of multidrug-resistant organism in the hospital setting.

The microbiological diagnosis relies on the isolation of potential pneumonia-causing microorganisms from pleural fluid, blood or a valid sample of respiratory secretions. These samples had to be obtained before the start of antibiotic therapy. Nonetheless, in no case this sample collection should ever result in a delay in the initiation of empirical treatment (29).

All patients suspected to have HAP should undergo these diagnostic tests:

1. **Blood cultures.** Two blood cultures will be drawn through two venipunctures performed under aseptic conditions with a minimum interval of 20 minutes between them. Despite to their low sensitive (less than 20%) and a positive isolation which does not confirm a pulmonary origin, they are recommended as they can help to identify responsible pathogens. They also inform the physician of the presence of other concomitant infections that are not directly related to pneumonia but may have prognostic implications.

As an example, *Candida* and *Enterococcus* species are not known to cause pneumonia. Therefore, identifying these pathogens in the bloodstream may direct guide to a separate and previously unsuspected site of infection such as a catheter-related bloodstream infection.

2. **Sample of respiratory secretion.** These samples can be obtained using invasive or non-invasive techniques, depending on the location of collection, whether it is distal or proximal, and the depth of the technique employed.

#### **Non-invasive Technique:**

- **Sputum samples:** Should be collected from non-intubated patients who can produce sufficient respiratory sputum. To evaluate the quality of such sputum, it must be representative of the lower airway according to **Murray-Washington's criteria (ANNEX 1)**. Optimal quality is achieved when there are >25 polymorphonuclear cells and <10 epithelial cells per low magnification field.

This technique is quick, easy and requires minimal resources with few complications. However, a potential disadvantage is that it may overestimate the presence of bacteria, leading to unnecessary antibiotic treatment and promoting antibiotic resistance.

#### **Invasive techniques:**

Bronchoscopy techniques are the preferred approach for intubated patients. Their advantages include a higher level of specificity in identifying the causative pathogen of respiratory infection more accurately, thus, reducing antibiotic overuse. Also, they allow for larger samples, which can facilitate further diagnostic testing if required.

However, their disadvantages include difficulty in performing them in non-intubated patients, higher cost, augmented risk of complications (such as pneumothorax or haemorrhage), and the need for qualified personnel to perform them. Moreover, the sensitivity and the precision of these procedures decrease significantly when they are performed on patients who have already begun antibiotic therapy.

- **Endotracheal aspiration (semi-quantitative or quantitative sputum)** involves aspirating respiratory secretions through the endotracheal tube, exclusively applicable to intubated patients. This technique enables the differentiation between bacterial colonisation and active respiratory infections (concentrations  $> 10^6$  CFU/mL).
- **Bronchial aspirate (BAS):** samples are obtained directly from the bronchi using bronchoscopy, an invasive technique. This method is equivalent to tracheal aspirate and requires a concentration of  $> 10^6$  CFU/mL to confirm active respiratory infection.
- **Bronchoalveolar lavage (BAL):** this technique consists of the introduction of a sterile saline solution of 150 mL (3 syringes of 50 mL each) into the bronchi through bronchoscope. Subsequently, the solution is recovered to obtain samples of cells and other components present in the bronchial tree and pulmonary alveoli. The first sample represents bronchial cellularity and the next, alveolar cellularity, which is sent for culture. It is considered that a pathogenic agent has an infective role of respiratory infection when it is found in BAL fluid in concentrations of  $>10^4$  CFU/mL.
- **Protected specimen Bronchial Brushing (PSB)** is a technique that involves introducing a brush protected with a polyethylene glycol cap through bronchoscopy for brushing the area of interest. It protects the collected cells and materials obtained from contamination from the upper airway by oropharyngeal flora. A sample is considered positive if it has a concentration of  $>10^3$  CFU/mL.

**Table 4:** Comparison of non-invasive and invasive diagnostic techniques (35)

| Type of sampling                           | Advantages   | Limitations   |
|--|--|---|
| <b>Non-invasive</b><br>(proximal sampling) | <ul style="list-style-type: none"> <li>- Fast and easy to perform</li> <li>- Cheap</li> <li>- Sensitive</li> <li>- No qualified personal needed</li> <li>- Less resources needed</li> <li>- Less potential complications</li> </ul>    | <ul style="list-style-type: none"> <li>- Higher risk of contamination with oral flora</li> <li>- Lower specificity</li> <li>- Might overestimate the presence of pathogens and lead to overtreatment</li> </ul>   |
| <b>Invasive</b><br>(distal sampling)       | <ul style="list-style-type: none"> <li>- Higher specificity</li> <li>- More accurate pathogen identification might help reduce antibiotic overuse</li> <li>- Large quantify of sample for complementary test when is needed</li> </ul> | <ul style="list-style-type: none"> <li>- Less sensitivity if antibiotic treatment is started</li> <li>- Qualified personnel needed</li> <li>- Higher cost</li> <li>- Difficult to perform in non-intubated patients</li> <li>- Higher risk for complications</li> </ul> |

Once respiratory secretions have been obtained, the microbiological results can be analysed as qualitative, quantitative or semi-quantitative information.

**Qualitative analysis** identifies pathogens presence (+) or absence (-) in the sample. However, its low positive predictive value (VPP) necessitates caution as it fails to detect the degree of colonisation, and tends to overestimate bacterial infection, leading to inappropriate antibiotic use and increased resistance.

In contrast, **quantitative assessment** establishes a threshold concentration of bacterial growth to differentiate between bacterial colonisation and active respiratory infection. When the concentration falls below this threshold, it is attributed to colonisation or contamination, leading to the diagnosis of HAP and the recognition of the pathogen as the cause of the infection. This analysis has the advantage that provides a useful way of detecting the presence of pneumonia and allows knowing the degree of microbial load in the sample. However, it also has the disadvantage of being susceptible to generating false negatives, which may result in the cessation of appropriate therapy ultimately leading to treatment failure.

Finally, **semi-quantitative results** describe bacterial growth as light, moderate, or severe. According to guidelines, non-invasive sampling with semi-quantitative cultures is the preferred method for diagnosing lower respiratory tract infections, as they reduce costs and minimise patient harm compared to quantitative-invasive tests.



The approach to respiratory specimen and culture selection for the microbiological diagnosis of nosocomial pneumonias differs between European and American guidelines. The 2017 European guideline (13) recommends obtaining distal quantitative respiratory samples before initiating antibiotic treatment in order to improve the accuracy of results and facilitate optimal treatment selection. In contrast, the 2016 American guideline (4) suggests the use of non-invasive techniques with semi-quantitative cultures over invasive techniques with quantitative cultures, referring that pathogen outcomes are equivalent and such techniques are significantly less invasive and harmful for patients. However, both recommendations are weak and supported by low-quality evidence, necessitating further research.

Additionally, regarding the importance of **direct examination** and **Gram stain** analysis from respiratory specimens is essential as it offers enhanced diagnostic support for nosocomial pneumonia, as a fast technique with results being available in less than 1 hour. However, current data are controversial, and their utility are limited by their sensibility and specificity. Although high-quality Gram Stain with numerous and predominant organisms may corroborate to HAP diagnosis, the absence of microorganisms in Gram stain cannot exclude pneumonia. For that reason, it is crucial to review culture results.

While cultures are still considered as the "gold standard" method for diagnosing and confirming the pathogen responsible for nosocomial pneumonia, the process is time-consuming. Furthermore, at times, inert, slow-growing, or non-cultivable pathogens may remain undetected using this method.

Finally, modern molecular diagnostic techniques have become increasingly important due to their ability to overcome limitations associated with conventional culture methods. Although molecular methods are essential diagnostic tools presently and, in the future, their substitution for traditional culturing techniques as the "gold standard" will require time and high-quality evidence from well-designed trials.

### 2.1.7 Therapeutic management

Therapeutic management of nosocomial pneumonia is divided into two parts: the initial **empirical treatment**, which is primarily based on five parameters: disease severity of the current illness, type and number of underlying disease and their severity, previously used antibiotics, risk factors of MDRO and local pattern of antimicrobial susceptibility. When the microbiological results are available, clinicians must implement **definitive treatment**, adjusting the empirical treatment to a targeted antimicrobial therapy to prevent the overuse of broad-spectrum antibiotics.

#### **Empirical treatment:**

Samples should be collected as soon as possible for microbiological studies in patients with suspected nosocomial pneumonia. However, the initiation of empirical treatment should not be delayed under any circumstances and should be started within the first hour after diagnosis. A fundamental aspect at this point is to ensure that such initial treatment is appropriate and adequate.

Appropriate empirical treatment involves administering the antibiotic to which the potential causative microorganisms are susceptible. Additionally, appropriate treatment consists of selecting an antibiotic at correct doses, with good penetration into the lung and, when indicated, in combination with other antibiotics.

The latest international guidelines in Europe and America (4,13) confirm that appropriate empirical treatment depends on the presence or absence of risk factors for MDRO. For this purpose, it is essential to know the most common pathogens in the hospital environment where the patient is admitted (local ecology) and the patient's previous microbiological data, if available (such as prior cultures).

For this reason, it is highly recommended that every hospital produces antibiograms to guide healthcare professionals in selecting the most effective antibiotics to minimise patient harm and exposure to unnecessary antibiotics, as well as to reduce the development of antibiotic resistance.

For patients treated in units with a low prevalence of MDRO or without high risk of mortality, we suspect of *Pseudomonas aeruginosa*, *S. Aureus* and other gram-negative bacilli, hence it is recommended to start **monotherapy** with a broad-spectrum antibiotic with antipseudomonal activity:

| Antibiotic (Antibiotic family)  | Dose   | Interval      | Infusion time |
|---|--------|---------------|---------------|
| <b>Piperacillin-Tazobactam</b><br>( $\beta$ -lactam with a $\beta$ -lactamase inhibitor)          | 4 g    | each<br>6-8 h | 2-3 hours     |
| <b>Ceftazidime or Cefepime</b><br>(3 <sup>rd</sup> and 4 <sup>th</sup> generation cephalosporins) | 2g     | each<br>8 h   | 2-3 hours     |
| <b>Levofloxacin</b><br>(anti-pneumococcal fluoroquinolone)  | 500 mg | each<br>12 h* | 1 hour        |

\*Administer this dose for 3 days and follow with 500 mg every 24 hours.

In case of patients with risk factors for MDRO, it is recommended to start **combined treatment** with broad-spectrum antibiotics with antipseudomonal activity:

| Antibiotic (Antibiotic family)  | Dose | Interval      | Infusion time |
|---|------|---------------|---------------|
| <b>Piperacillin-Tazobactam</b><br>( $\beta$ -lactam with a $\beta$ -lactamase inhibitor)          | 4 g  | each<br>6-8 h | 2-3 hours     |
| <b>Ceftazidime or Cefepime</b><br>(3 <sup>rd</sup> and 4 <sup>th</sup> generation cephalosporins) | 2g   | each<br>8 h   | 2-3 hours     |

| Antibiotic (Antibiotic family)                              | Dose            | Interval     | Infusion time |
|---|-----------------|--------------|---------------|
| <b>Levofloxacin</b><br>(anti-pneumococcal fluoroquinolone)  | 750 mg          | each<br>24 h | 1 – 1 ½ hours |
| <b>Ciprofloxacin</b><br>(anti-pneumococcal fluoroquinolone) | 400 mg          | each<br>8 h  | ½ hour        |
| <b>Amikacin</b><br>(aminoglycoside)                         | 15 -20<br>mg/kg | each<br>24 h | ½ - 1 hour    |
| <b>Tobramycin</b><br>(aminoglycoside)                       | 5-7<br>mg/kg    | each<br>24 h | ½ - 1 hour    |
| <b>Aztreonam</b><br>( $\beta$ -lactam - Monobactam)         | 2 g             | 8 h          | ½ - 1 hour    |

In case of risk factors for MRSA, add:

MRSA coverage should only be initiated if the patient has received intravenous antibiotics in the last 90 days, is hospitalised to a unit where at least 20% of MRSA, the prevalence is unknown or is at high mortality risk. The choice of the antibiotic will be conditioned by host factors such as renal function, use of serotonin reuptake inhibitors, hemogram, etc.

| Antibiotic ( <i>Antibiotic family</i> )   | Dose     | Interval    | Infusion time |
|---|----------|-------------|---------------|
| <b>Linezolid</b> ( <i>oxazolidinone</i> ) | 600 mg   | each 12 h   | 1 hour        |
| <b>Vancomycin</b> ( <i>glycopeptide</i> ) | 15 mg/Kg | each 8-12 h | 1-3 hours     |

In case of suspicion of ESBL:

| Antibiotic ( <i>Antibiotic family</i> )                    | Dose      | Interval   | Infusion time |
|--|-----------|------------|---------------|
| <b>Ertapenem</b> ( <i>carbapenem non-antipseudomonal</i> ) | 1g        | each 24 h  | ½ hour        |
| <b>Meropenem</b> ( <i>Carbapenem</i> )                     | 500mg -1g | each 6-8 h | 2-3 hours     |
| <b>Imipenem</b> ( <i>Carbapenem</i> )                      | 500 mg    | each 6 h   | 1 hour        |

Causes of lack of response to empirical treatment:

There are numerous factors that may lead to rapid deterioration or lack of improvement to empirical treatment in patients suspected of having nosocomial pneumonia. The main known causes are summarised in the following table:

**Table 5:** Causes of lack of response to empirical treatment. Extracted from (5).

| Microorganisms or antibiotics   | Other infections  | No Infections  | Host factors   |
|---|---|--|--|
| <ul style="list-style-type: none"> <li>- Inappropriate antibiotic choice or combination</li> <li>- Low dose of antibiotic</li> <li>- Antibiotic Resistance</li> <li>- Microorganisms outside the usual spectrum</li> <li>- Super infection</li> </ul> | <ul style="list-style-type: none"> <li>- Sinusitis</li> <li>- Catheter-associated sepsis</li> <li>- Abdominal sepsis</li> <li>- Urinary sepsis</li> <li>- Pulmonar Absces</li> <li>- Pleural Effusion</li> <li>- Empyema</li> </ul> | <ul style="list-style-type: none"> <li>- Acute respiratory distress syndrome</li> <li>- Atelectasis</li> <li>- Pulmonar hemorrhage</li> <li>- Pulmonar embolism</li> <li>- Hear failure</li> <li>- Pulmonar contusion</li> <li>- Post-pulmonary resection edema</li> <li>- Drug's fever</li> </ul> | <ul style="list-style-type: none"> <li>- Advanced age</li> <li>- Prolonged mechanical ventilation</li> <li>- Severe respiratory failure</li> <li>- Chronic pulmonar disease</li> <li>- Incresed systemic inflamatory response</li> </ul> |

**Definitive treatment:**

When microbiological results are obtained, a modification to the empirical treatment may be necessary if resistant or unexpected pathogens are isolated in a patient who does not respond to the treatment. In contrast, in cases of low clinical suspicion and absence of pathogen identification, cessation of antibiotic should be contemplated. Consequently, therapeutic de-escalation or treatment reduction may be implemented, via the following measures:

- Utilization of narrow-spectrum agents to patients infected with susceptible strains.
- Ceasing anti-MRSA antibiotics if MRSA is not detected.
- Limiting the use of carbapenems to pathogens that are only susceptible to this class of antibiotics, specifically ESBL-producing *Enterobacteriaceae*, *Pseudomonas* and *Acinetobacter spp.*

After identifying the pathogens responsible for the infection and acquiring susceptibility results, the antibiotic treatment will be de-escalated based on resistance/sensitivity profile, always choosing an antibiotic with good penetration into lung tissue and the narrowest antimicrobial spectrum. The therapeutic de-escalation strategy has proven effective in reducing overall antibiotic utilisation without a significant increase in the recurrence or mortality rates. Combination therapy is mainly useful in increasing the likelihood of appropriate treatment rather than improving patient prognosis. However, when appropriate definitive treatment has been made, the non-de-escalation of empirical treatment will only contribute to the development of multidrug resistance.

**Duration of treatment:**

The optimal duration of antibiotic treatment has been a topic of discussion in recent guidelines, aiming to maintain the antibiotic treatment long enough to eliminate the infection but not unnecessary extending it to avoid adverse effects, changes in the flora's patient and/or the development of multidrug resistance.

While previous guidelines recommended for a duration up to 14 days or more, current recommendations have stated that a duration of 7-8 days is appropriate for patients with HAP with good clinical response. This length of treatment has shown no increase in relapses or mortality rates, in comparison to longer treatments.

Furthermore, using this approach has contributed to a decrease in multi-drug resistant pathogens. For patients with risk factors for multidrug resistance, longer antibiotic regimens up to 14 days may be necessary in case of inadequate initial response or complications such as lung abscess, necrotising pneumonia, or pleural effusion. Treatment always should be individualised according to the patient's clinical response.

### 2.1.8 Prevention

There are multiple strategies available to prevent nosocomial pneumonia. Implementation of these strategies is equally important as accurate diagnosis and treatment. Below, there is a concise list of generally recommended measures to prevent nosocomial pneumonia: (4,5,36,37)

1. Hand disinfection using solutions by healthcare staff must take place before and after patient contact to maintain optimal hygiene standards.
2. Recommendation to use masks and tissues for patients with a cough can reduce the transmission of respiratory pathogens.
3. Oral hygiene with use of chlorhexidine oral cleansing, electric toothbrushing and oral hygiene instruction in hospital units should also be optimised (38).
4. Monitoring and early removal of invasive devices:
  - Early extubating
  - Preference for non-invasive mechanical ventilation
  - Avoid endotracheal intubation/reintubation
5. Tight control of sedation medication.
6. Early identification and treatment of dysphagia, especially in the elderly and in patients with recent stroke or surgical procedures.
7. Aspirating subglottic secretions and stimulation of cough and deep breaths to improve secretion clearance and lung volume.
8. Enteral nutrition is preferred over parenteral nutrition to reduce the risk of complications associated with intravenous catheters and to prevent villous atrophy of the intestinal mucosa, which can increase the risk of bacterial translocation.
9. Elevating the head of the bed to a 30° - 40° position with the patient semi-recumbent.
10. Giving preference to the sitting position for patients.

11. Encouraging early ambulation.
12. Avoid unnecessary intrahospital transfers and unnecessary manipulations of medical equipment.
13. Maintenance of ventilator circuits in accordance with the recommendations for sterilisation and disinfection for respiratory care equipment should be carried out to reduce the risk of circuit contamination.
14. Pharmacological prophylaxis should be considered for deep vein thrombosis. Prophylaxis for stress ulcer is not indicated for HAP prevention, so prescribing Proton-pump inhibitors (PPI) and H<sub>2</sub>-receptor blockers should depend on the patient's risk of gastrointestinal bleedings.

Other Considerations:

- Selective digestive decontamination with a topical antiseptic administered enterally for up to 5 days to prevent HAP and VAP. A recent French study (39), demonstrated a decrease in mortality as well as a reduction in the acquisition of MDRO. However, this indication may be limited in units that already have high prevalence of MDRO.
- Probiotics and antibiotics are still under evaluation to determine their efficacy in preventing nosocomial pneumonia. In theory, probiotics could potentially reduce the incidence of HAP by improving intestinal barrier function, increasing host cell antimicrobial peptides, and regulating the composition of the intestinal flora (40).

## 2.2 The development of Molecular Techniques

Several molecular diagnostic techniques have been developed over the last years to identify the microorganism and determine antibiotic susceptibilities, as well as potential determinants of antimicrobial resistance in patients with nosocomial pneumonia, within few hours of sample collection, by detecting and amplifying the pathogen genome.

These molecular diagnostic methods that utilise polymerase chain reaction (PCR) to identify pathogen DNA can provide significant advantages over conventional culture-based methods. First of all, they provide rapid results, with outcomes available within one hour, compared to traditional techniques that require a waiting period of at least 72 hours. This swiftness confers considerable benefits in reducing the incidence of multi-drug resistant organisms and ultimately decreasing the need for broad-spectrum and unnecessary antibiotics. Also, it demonstrates a high sensitivity and specificity rates and the ability to identify microorganism undetectable by culture (for example, viruses).

Additionally, the use of the new tools that utilise multiplex PCR (FilmArray) directly applied to fresh samples may provide benefits, such as enabling early de-escalation and narrowing of antimicrobial treatment in specific scenarios such as the withdrawal or withholding of anti-MRSA antibiotics (41).

Nevertheless, despite all the potential benefits that this new technique has to offer, their application is still limited and usually it must be accompanied by other diagnostic test due to the lack of bacterial quantification, which is crucial to distinguish between colonising microorganisms and true pathogens, which potentially leads to false-positive test and could lead to an over-use of unnecessary antibiotics (42). Moreover, molecular techniques demand specific expertise and high operational expenses that are not accessible among all hospitals.

### 2.2.1 Film Array

The Film Array technique is a diagnostic approach that uses multiplex PCR to detect and identify multiple pathogens, including bacteria and viruses, as well as antimicrobial resistance genes, in an unprocessed clinical sample. That provides an aetiological diagnosis of nosocomial pneumonia in approximately one hour, allowing for a much more targeted treatment for the patient.



### 2.2.2 FilmArray Pneumonia *Plus* Panel

The FilmArray Pneumonia *Plus* Panel is a multiplex nucleic acid test designed to detect and identify multiple respiratory viral and bacterial nucleic acids, as well as select antimicrobial resistance genes, in sputum specimens (induced or expectorated sputum) or bronchoalveolar lavage specimens obtained from patients with suspected lower respiratory tract infections.

This system specifically analyses **semi-quantitatively** 15 bacteria (11 gram-negative and 4 gram-positive) and **qualitatively** 3 atypical bacteria, 9 virus and 7 antibiotic resistance markers (43).

**Table 6:** BioFire FilmArray Pneumonia *Plus* Panel targets

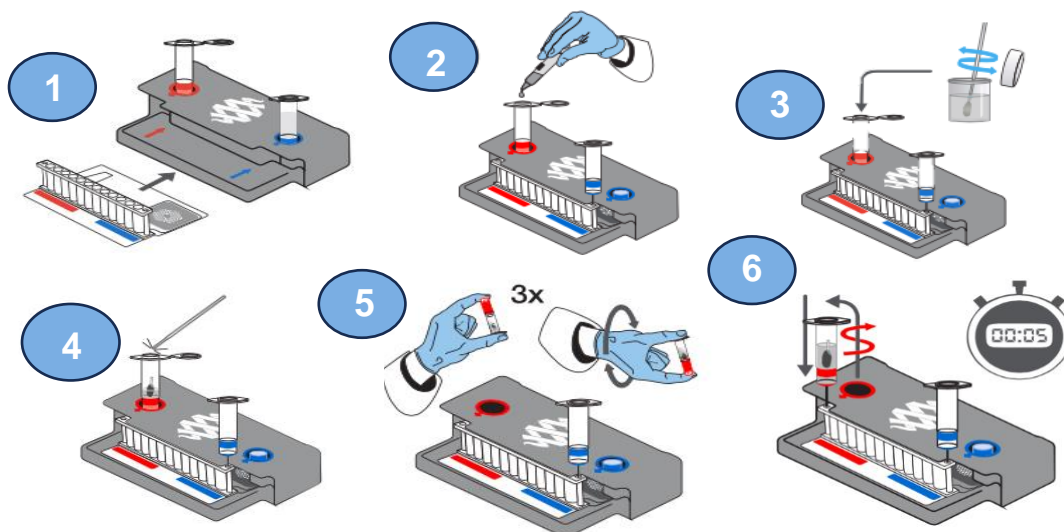
|                                  |   |
|----------------------------------|---|
| 15 BACTERIA                      | <ul style="list-style-type: none"> <li>- <i>Acinetobacter baumannii</i> complex</li> <li>- <i>Enterobacter cloacae</i></li> <li>- <i>Escherichia coli</i></li> <li>- <i>Haemophilus influenzae</i></li> <li>- <i>Klebsiella aerogenes</i></li> <li>- <i>Klebsiella oxytoca</i></li> <li>- <i>Klebsiella pneumoniae</i> group</li> <li>- <i>Moraxella catarrhalis</i></li> <li>- <i>Proteus</i> spp</li> <li>- <i>Pseudomonas aeruginosa</i></li> <li>- <i>Serratia marcescens</i></li> <li>- <i>Staphylococcus aureus</i></li> <li>- <i>Streptococcus agalactiae</i></li> <li>- <i>Streptococcus pneumoniae</i></li> <li>- <i>Streptococcus pyogenes</i></li> </ul> |
| 3 ATYPICAL BACTERIA              | <ul style="list-style-type: none"> <li>- <i>Chlamydia pneumoniae</i></li> <li>- <i>Legionella pneumophila</i></li> <li>- <i>Mycoplasma pneumoniae</i></li> </ul>  |
| 9 VIRUSES                        | <ul style="list-style-type: none"> <li>- Adenovirus</li> <li>- Coronavirus</li> <li>- Human Metapneumovirus</li> <li>- Human Rhinovirus</li> <li>- Human Enterovirus</li> <li>- Influenza A Virus</li> <li>- Influenza B Virus</li> <li>- Parainfluenza Virus</li> <li>- Respiratory Syncytial Virus</li> </ul>   |
| 7 ANTIMICROBIAL RESISTANCE GENES | <ul style="list-style-type: none"> <li>- MRSA genes (<i>mecA/C</i> and <i>MREJ</i>)</li> <li>- ESBL (<i>CTX-M</i>)</li> <li>- Carbapenemases (<i>KPC</i>, <i>NDM</i>, <i>OXA-48-like</i>, <i>VIM</i>, <i>IMP</i>)</li> </ul>  |

The system combines sample preparation, nucleic acid extraction and purification, amplification, detection, and analysis in a single equipment that necessitates only two minutes of manipulation, obtaining a result in about an hour. (44) This diagnostic method for detecting pathogens responsible for HAP in respiratory sputum samples has demonstrated a sensitivity and specificity of 96.3% and 97.2%, respectively (45).

### **The procedure for the FilmArray Multiplex PCR System:**

#### **STEP 1: Sample preparation.**

- **Prepare and hydrate pouch:** Insert the cartridge into the pouch loading station. [1] Place the sample injection vial in the red well and the hydration injection vial in the blue well. Later, insert the hydration injection vial into the hydration port of the cartridge and push down till is drawn into the cartridge. [2]
- **Prepare sample mix:** Dispense sample buffer into the sample injection vial slowly but with forceful squeeze. Use the sample swab to stir the entire specimen for about 10 seconds. [3] Place the swab end into the sample injection vial and break off the swab handle. [4] Finally, invert the sample injection vial 3 times and then return to the red well of pouch loading station. [5]
- **Load sample mixt:** Unscrew the sample injection vial from the cap, wait 5 seconds to reduce the risk of contamination and then remove the sample injection vial. [6] Insert the sample injection vial into the pouch sample port and wait till is all drawn. The pouch is now ready for analysis.

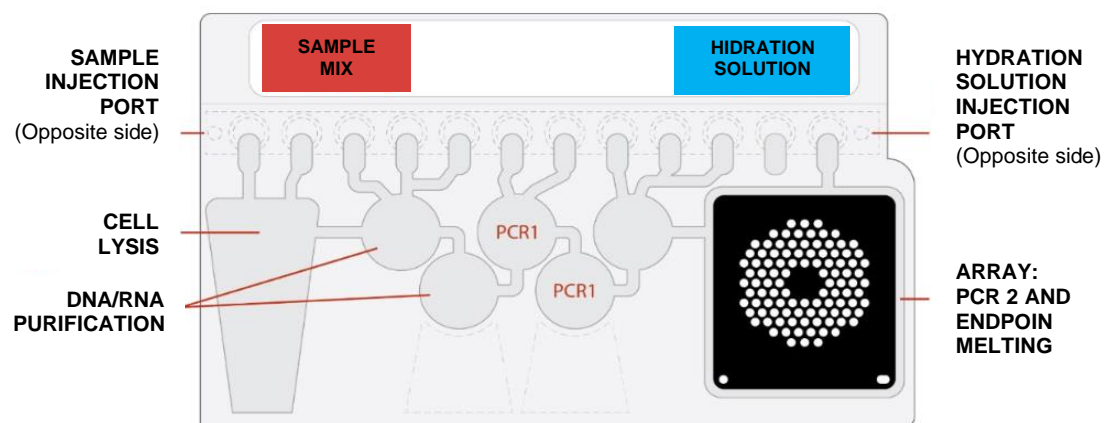


**Figure 2:** Steps to follow for sample preparation. Image adapted from (46)

**STEP 2: FilmArray setup and analyse.**

Now, the sample is inside the FilmArray instrument, and it is ready to analyse. First, the sample is moved into the lysis chamber, where the organisms are agitated at high speed to break up all cells and viruses, releasing the nucleic acid. This process is known as bead beating. These nucleic acids are moved from the lysis chamber to the purification chamber by magnetic beads.

At this stage, residual cellular and viral remnants are eliminated by employing a wash buffer. The FilmArray activates a magnet outside the pouch, which holds the magnetic beads in place while the debris are washed away. Subsequently, an elution buffer facilitates the liberation of 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.



**Figure 3:** Functioning of the FilmArray panel. Image adapted from (46)

In the first PCR chamber, a reverse transcription process is executed to convert any target RNA into DNA, where many reactions simultaneously occur. Then, the products of this stage are diluted to limit any remaining first-stage PCR primers. All these products are combined with a fresh master mix and then, aliquoted to each well in the array.

Following, each well of the array is preloaded with a specific set of second-stage PCR primes, amplifying sequences present in the products generated during the first-stage PCR, with the aim of eliminate non-specific products commonly associated with conventional multiplex PCR methods.

A fluorescent double-stranded DNA blinded dye is used to monitor each reaction, because the second-stage primers only amplify target DNA to detect one specific target.

Finally, the identification of organisms relies on the determination of positive wells within the array. The FilmArray conducts a melting analysis to verify the existence or non-existence of distinctive temperature profiles associated with the second-stage PCR products.

**STEP 3: Get the results and analyse them.**

The FilmArray software processes all data and generates a positive or negative result for each organism. These outcomes are presented in a comprehensible report, indicating the detection status of each target within the sample.

## 2.3 Antimicrobial Resistance

### 2.3.1 Definition

Antibiotic resistance has always existed for millions of years due to mutations and gene transfer being part of human evolution. Nevertheless, in recent years, antimicrobial resistance rapidly emerges at a global scale and spreads from one country to the other faster than previously through, being endemic in many parts of the world. Additionally, to reduce the risk of treatment failure caused by antimicrobial resistance, broad-spectrum antibiotics are often prescribed empirically. This practice increases the likelihood of matching the administered antibiotic with the pathogen's susceptibility. Paradoxically, this approach may favour the development of MDRO.

Antibiotic resistance occurs when bacteria have or develop the ability to circumvent the mechanism, which drugs use against them. Infections caused by antibiotic-resistant pathogens are typically more challenging to treat and can relapse and cause significant morbidity and mortality (47). Globally, approximately 700.000 deaths are attributed to antimicrobial resistance annually, and these numbers could rise to 10 million deaths per year by 2050 (48). Apart from that, infections due to antimicrobial-resistant bacteria result in longer duration of hospitalisation and pose a significant economic expense on the national healthcare system.

This antibiotic resistance exhibited by bacteria can be intrinsic, acquired or adaptive: **Intrinsic** resistance is defined as the resistance generated due to the inherent properties of the bacterium. **Acquired resistance** happens when a previously sensitive microorganism acquires a resistance mechanism by either a mutation or the acquisition of new genetic material from an exogenous source with horizontal gene transfer through three main mechanisms (transformation, transduction and conjugation). Finally, **adaptive resistance** is defined as the resistance to one or more antibiotic induced by a specific environmental signal (e.g. stress, growth state, pH, concentrations of ions...). It is transient and generally reverts to the original state once the inducing signal is removed (47).

In addition to the basic concept of antibiotic resistance, the terms **multidrug-resistant (MDR)**, **extensively drug-resistant (XDR)** and **pandrug-resistant (PDR)** are used. MDR refers to a microorganism that is resistant *in vitro* to at least one antibiotic in three or more antibiotic classes, XDR describes bacteria that are susceptible to only one or two antibiotic class and PDR when it is resistant to all available antibiotic classes (49).

### 2.3.2 Mechanisms antibiotic resistance

There are different mechanisms which microorganisms might exhibit resistance to drugs, and they can be summarised into these 4 types of resistance mechanisms:

1. Drug inactivation or modification through production of enzymes that either destroy or alter the antibiotic, rendering it ineffective.
2. Decreased drug accumulation by either decreased outer membrane permeability or increased active efflux pumps of the drugs across the cell surface, preventing or hindering the action of the antimicrobial.
3. Alteration of target or binding sites such alteration of penicillin-binding proteins or alteration of ribosomal-binding proteins.
4. Alteration of metabolic pathways that bypasses the reaction inhibited by the drug, such as the ability of enterococci to absorb folic acid from the environmental, which allows them to bypass the effects to trimethoprim-sulfamethoxazole.

Microorganisms seem to have developed a preference for certain types of resistance mechanisms over others. For example, gram-negative bacteria tend to rely on the production of beta-lactamase enzymes to destroy penicillin, while gram-positive bacteria tend to modify the penicillin-binding sites to make them resistant to the penicillin antibiotic (50).

#### **Extended spectrum $\beta$ -lactamase (ESBL):**

$\beta$ -lactamase is an important mechanism of resistance in gram-negative bacteria. These enzymes hydrolyse the  $\beta$ -lactam ring of  $\beta$ -lactam antibiotics, rendering them ineffective. ESBL confer resistance to a broader array of antibiotics, including, in addition to penicillins, beta-lactams with extended coverage such as 3<sup>rd</sup> generation cephalosporins, and the monobactam aztreonam.

For example, *Klebsiella* is intrinsically resistant to ampicillin and other penicillins and can acquire resistance to cephalosporins and aztreonam by the production of extended-spectrum  $\beta$ -lactamases (ESBLs).

- **CTX-M:** CTX-M is a class A extended-spectrum  $\beta$ -lactamase which confers resistance to a broad spectrum of cephalosporins. This group of  $\beta$ -lactamases can be plasmid-borne and the *bla*CTX-M gene may be found in multiple copies per cell within a variety of gram-negative hosts. Phylogenetic analyses of CTX-M describe 5 phylogroups and over 150 types or variants. CTX-M ESBLs are predominantly found in the *Enterobacteriaceae* family. However, they have also been reported in other non-enteric gram-negative bacteria such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Vibrio* spp. and *Aeromonas* spp.

#### **MRSA (Methicillin Resistance):**

Methicillin-resistant *Staphylococcus aureus* (MRSA) produces a penicillin-binding protein with reduced affinity for  $\beta$ -lactam antibiotics. Without the ability to penetrate into the cell, the antibiotic is ineffective. That is encoded by the *mecA* gene and strains with that gene are resistant to all commercially available  $\beta$ -lactams and many other anti-staphylococcal drugs (51).

- **MecA/C and MREJ:** Methicillin-resistant *staphylococci* are a serious concern in both HAP and CAP. Few options exist for treatment of these infections, as the bacteria are resistant to both natural and semi-synthetic  $\beta$ -lactam antibiotics. The primary mechanism of methicillin resistance is through acquisition of the *mecA* gene that encodes a penicillin binding protein (PBP2a) that has low affinity for  $\beta$ -lactams. The *mecA* gene is carried on a chromosomally-integrated mobile genetic element called the staphylococcal cassette chromosome *mec* (SCC*mec*). In *S. aureus*, the *mec* cassette integrates into a specific region in the *S. aureus* genome; this insertion creates MREJ (SCC*mec* right-extremity junction). The junction, or point of insertion of *mecA/C* in the cassette, can vary leading to a variety of MREJ types.

**Carbapenem resistance:**

Carbapenem resistance can develop through various mechanisms, and one of the most common one is the production of carbapenemases, which are enzymes that can hydrolyze the beta-lactam ring of carbapenems and other beta-lactam antibiotics.

*Pseudomonas aeruginosa* is the most common MRD gram-negative bacterial pathogen causing HAP. It has intrinsic resistance to many antimicrobial agents but in some strains, IMP-type enzymes, which confer the acquisition  $\beta$ -lactamases activation against carbapenems, antipseudomonal penicillins and cephalosporins, raising a big concern in this regard.

Following, *Acinetobacter* species are generally less virulent than *P. aeruginosa*, but their increasing resistance to commonly used antimicrobial agents has become a general worry. 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.

Finally, *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 which have significant toxicity and efficacy limitations.

- **KPC:** The *Klebsiella pneumoniae* carbapenemase (KPC) gene, confers resistance to the carbapenem class of  $\beta$ -lactams and currently is thought to be the most common and rapidly emerging carbapenemase around the world. Though originally isolated from *Klebsiella pneumoniae*, the gene has since disseminated to other species including *Acinetobacter*, *Pseudomonas*, *Enterobacter*, *Serratia*, *Salmonella*, *Escherichia coli*, *Klebsiella oxytoca*, and other *Enterobacteriaceae*. There are several known KPC variants but the most commonly isolated types are KPC-2 and KPC3.
- **NDM:** The New Delhi metallo- $\beta$ -lactamase (NDM) is a plasmid-mediated enzyme that confers resistance to all current  $\beta$ -lactam antibiotics, with the exception of aztreonam. There are 16 different NDM types that may be found in a variety of gram-negative species. The *bla*NDM gene is widely



and rapidly disseminated throughout the *Enterobacteriaceae*, as well as other gram-negative bacteria. The plasmids encoding NDM are easily transferable and capable of wide rearrangement, suggestive of extensive transmission, as well as plasticity, amongst bacterial populations. Multi-drug resistant NDM-producing bacteria are now the most prevalent carbapenemase producers in Europe, and this trend is expected to continue worldwide.

- **IMP:** Imipenem  $\beta$ -lactamases are plasmid-borne metallo- $\beta$ -lactamases (MBLs) belonging to Ambler class B1 MBLs. Many distinct IMP types have been identified (numbered 1-60), which have the potential to confer different levels of antibiotic resistance to broad-spectrum  $\beta$ -lactams like carbapenems, cephamycins, and cephalosporins. MBLs hydrolyze almost all  $\beta$ -lactams, rendering ineffective products, resulting in bacterial resistance to this class of antibiotics. Carriage of a *bla*IMP gene has been detected in strains of *Serratia marcescens*, *Klebsiella pneumoniae*, *Pseudomonas*, *Escherichia coli*, and *Enterobacter cloacae*. Due to recent recommendations to treat CAP with  $\beta$ -lactams, the increased development of MBL resistance in lower respiratory infections is a particular concern.
- **OXA-48-like:** The oxacillinase (OXA)  $\beta$ -lactamases are a group of primarily plasmid-mediated enzymes that confer resistance to penicillins, cephalosporins, and carbapenems. The *bla*OXA-48 gene, and several OXA-48-like variants have been identified in various gram-negative bacteria in the *Enterobacteriaceae* family. OXA-48 hydrolyzes penicillins at a high level, carbapenems at a low level with greater activity against imipenem than meropenem, and demonstrates very weak activity against expanded-spectrum cephalosporins.
- **VIM:** Verona Integron-Encoded Metallo- $\beta$ -Lactamase (VIM) are integron-encoded carbapenemases. There are reports of both plasmid and chromosomal localization of the *bla*VIM integron; however, the majority of *bla*VIM alleles are found on plasmids. There are approximately distinct 50 types of VIM types. VIMs are found mainly in gram-negative bacteria, including enteric bacteria, with a vast majority associated with various species of genus *Pseudomonas*.

### 3. JUSTIFICATION

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Nosocomial pneumonia is one of the most frequent infections acquired among hospitalised patients. Such infections have a significant impact on patients, increasing hospital stay, morbidity, mortality, and health care costs, especially when such infections are caused by multidrug-resistant pathogens (4).

Related to that, the WHO has long been warning about this major health problem worldwide, characterising it as the “pandemic of antibiotic resistance” and identifying it as one of the primary threats to human health in the coming years. WHO experts affirm that if things do not change, by 2050, antibiotic resistance will be the leading cause of mortality worldwide, responsible for over 10 million annual deaths (48).

Currently, diagnostic methods considered the "gold standard" require at least five days to identify the causative pathogen of pneumonia. During this time, empirical treatment with broad-spectrum antibiotics is prescribed to attempt to cover the most common pathogens causing pneumonia, without knowing the true aetiology with certainty. This practice often leads to an increased risk of acquiring MDRO, as incorrect or inadequate treatment is frequently administered to the patient.

To this big problem, it must be added that many physicians, once they have the microbiological result, often do not reduce the broad-spectrum therapy, prioritising other aspects of the patient’s treatment, fearing a relapse of clinical deterioration.

Diagnostic molecular techniques, such as FilmArray rapid multiplex PCR, have successfully addressed the problem of delayed organism identification and antimicrobial susceptibility testing. By providing results in hours instead of days, these techniques have significantly reduced the time between identification and treatment initiation.

These statements can be based on data from the Santa Caterina laboratory database from November 2022 to December 2023 (**ANNEX 2**), which shows that a total of 62 FilmArray Respiratory diagnostic tests were performed, and 40 of which had positive results. Of these, only 21 detected bacteria and 3 of them

showed antibiotic resistance. Of the remaining 20, 7 detected viruses and 13 showed viral and bacterial co-infection. It can, therefore, be concluded that many patients received incorrect treatment.

It should also be noted that the use of the FilmArray Pneumonia *Plus* Panel is currently very restrictive in the hospital where the study was conducted. It is only indicated to clinicians in three situations: VAP patients with septic shock, CAP patients with septic shock, and immunocompromised or haematological patients on active chemotherapy. This restriction is due to the high cost of the test, around 175 €, despite clear clinical indications.

Limited data exists on whether the use of this technique improves antimicrobial stewardship or confers advantages in reducing antimicrobial resistance. Furthermore, most studies have focused on the impact of implementing this molecular technique in the ICU, with limited data on the potential impact of implementing it in internal medicine patients.

The aim of this study is to determine whether the application of FilmArray for diagnosing nosocomial pneumonia enables prompt targeted antibiotic treatment, thereby reducing the incidence of multidrug-resistant organism acquisition, improving clinical outcomes for patients and the major health problem of antibiotic resistance.

## 4. HYPOTHESES

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The assumptions that led to the start of this study are as follows:

### 4.1 Main hypothesis:

The use of the FilmArray Pneumonia *Plus* Panel, compared to bacterial culture, leads to a long-term reduction in bacterial antibiotic resistance in patients diagnosed with nosocomial pneumonia.

### 4.2 Secondary hypothesis.

- The use of the FilmArray Pneumonia *Plus* Panel for diagnosing the aetiology of nosocomial pneumonia will support the resolution of the infection and reduce hospital stay, related complications, readmissions to hospital and mortality rates.

## 5. OBJECTIVES

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The proposed project purposes the following objectives:

### 5.1 Main objective

The aim of this study is to assess whether the implementation of molecular diagnostic technique (FilmArray Pneumonia *Plus* Panel) and the subsequent targeted treatment for patients with nosocomial pneumonia, as opposed to conventional culture and empirical treatment, will result in a long-term reduction in bacterial antibiotic resistance.

### 5.2 Secondary objectives

- To compare complications in patients treated according to FilmArray molecular results with those treated according to conventional culture results.
- To compare the length of hospital stay in patients treated according to FilmArray molecular results with those treated according to conventional culture results.
- To compare hospital readmissions between patients treated based on FilmArray molecular results and those treated based on conventional culture results.
- To compare mortality rates between patients treated based on FilmArray molecular results and those treated based on conventional culture results.

## 6. MATERIAL AND METHODS

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### 6.1 Study design

This project is a **prospective, single-center, quasi-experimental study**. It will be a before-and-after evaluation of an intervention to improve bacterial resistance to antibiotics at Santa Caterina Hospital. The intervention consists of the implementation of the FilmArray Pneumonia *Plus* Panel in patients with nosocomial pneumonia.

#### **Period before intervention:**

Participants will provide a sputum sample for conventional bacterial culture, which is the gold standard technique for diagnosing nosocomial pneumonia. Empirical treatment will be initiated until culture results are available. If the results indicate that the current therapy is inappropriate, the treatment will be confidently modified to a targeted approach, involving de-escalation or modification of such treatment.

#### **Period after intervention:**

For participants assigned after the intervention, sputum samples will be collected to perform FilmArray. The FilmArray Pneumonia *Plus* Panel detects 18 bacteria, 9 viruses and 7 genetic markers of resistance. The analyser units will be located in the Clinical Laboratory of Microbiology and Parasitology of Santa Caterina Hospital. For these patients, treatment decisions will be guided based on the results obtained within one hour using the FilmArray, according to the established recommendations of the current guidelines.

### 6.2 Study population

The population of this study will be adult patients (>18 years) admitted to Hospital Santa Caterina in Salt, diagnosed with nosocomial pneumonia. Participants must meet the inclusion criteria and have no reason for exclusion, as indicated below.

#### **6.2.1 Inclusion criteria**

- Any patient hospitalised for >48 hours and:
  - o Aged 18 years or older.
  - o With criteria of nosocomial pneumonia:
    - New lung infiltrate on a Chest X-Ray .

- Evidence that the infiltrate is due to an infectious origin with compatible clinical (new onset of fever and/or purulent sputum and/or leukocytosis and/or decline in oxygenation).
- Dated and signed informed consent (**ANNEX 5**).
- Able to provide sufficient volume of sample from the lower respiratory tract for performing microbiological culture and FilmArray.

### 6.2.2 Exclusion criteria

- Pregnant women.
- Immunocompromised patients (chemotherapy or radiotherapy in the previous 90 days, use of immunosuppressive drugs, chronic use of corticosteroids at a minimum dose of 15 mg/day in the last 2 weeks, hematopoietic progenitor transplant, solid organ transplant and HIV with  $CD \leq 200$  cells/mm<sup>3</sup>).
- Imminent death (life expectancy  $\leq 24$  hours).
- Limitation of therapeutic effort (LTE).
- Radiological evidence of thoracic empyema or pulmonary abscess.
- Patients requiring antibiotic treatment for other indications.
- Classified unfit by the study investigator.
- Patients who were readmitted to the hospital.

### 6.2.3 Withdrawal criteria

Participants in the study are expected to follow the established follow-up protocol. Every effort should be made to ensure patients complete the study, unless there are justified reasons such as:

- **Death**
- **Follow-up loss**, which occurs when patients discharge themselves from the internal medicine ward to which they have been admitted or even request voluntary discharge.
- **Request for withdrawal of consent for the study (ANNEX 6)**: The participant may voluntarily decide to be excluded from the study. Whenever possible, this information should be obtained in written form.

Any loss of patients must be formally reported and documented, including the associated data and the reason for withdrawal. All data collected prior to the participant's withdrawal will be used for the study results.

## 6.3 Sample

### 6.3.1 Sample size

The sample size was estimated using the GRANMO Sample Size Calculator, and the setting for two independent proportions. Data collection from Santa Caterina laboratory database (**ANNEX 2**), demonstrate that the proportion of patients with MDRO is approximate 15%. To reduce and reverse the emergence of bacterial resistance to antibiotics, it is expected that the proportion of patients with MDRO after implementing the Film Array would be 2%.

In a two-sided test, accepting an alpha risk of 0,05 and a beta risk of 0,20%, a total of 37 subjects in the first group (pre intervention) and 111 subjects in the second group (post intervention) are estimated to be required to find a statistically significant difference in proportions. A drop-out rate of 10% has been anticipated.

### 6.3.2 Sample selection

All patients included in the study will be recruited from Santa Caterina Hospital in Salt. Patients who have been diagnosed with HAP and meet the inclusion criteria will be eligible for participation in the study. For this reason, a non-probabilistic consecutive recruitment method will be used in this study.

### 6.3.3 Study duration

As pneumonia typically has a higher incidence in the spring and winter months, extending the patient recruitment window over a 12-month period to cover all seasons will ensure comprehensive data collection and identify any seasonal trends.

Considering the epidemiological data indicating an annual incidence of approximately 50 reported cases of HAP per year in our hospital, the project is expected to take approximately 3 years to reach the required sample size for the study.

## 6.4 Variables and measurements

### **INDEPENDENT VARIABLES:**

- **Implementation of FilmArray:** in this study, the independent variable is the application of FilmArray Pneumonia *Plus* Panel in patients diagnosed with nosocomial pneumonia in the hospital. The technique is a multiplex PCR that detects and identifies multiple pathogens in an unprocessed clinical sample, providing an aetiological diagnosis of the pneumonia in approximately one hour in order to provide an appropriate early targeted antibiotic treatment. This system specifically analyses semi-quantitatively 15 bacteria (11 gram-negative and 4 gram-positive) and qualitatively 3 atypical bacteria, 9 virus and 7 antibiotic resistance markers.

The study variable is a dichotomous nominal qualitative variable, defined as 'yes' to the implementation of FilmArray or 'no' if the molecular diagnostic technique is not implemented.

### **DEPENDENT VARIABLES:**

#### - Main outcome:

- **Acquisition of Multidrug Resistant Organism:** this variable is defined as resistance in vitro to at least one antibiotic in three or more antibiotic classes. The diagnostic technique that is implemented in the study can measure such resistance, evaluating 7 antimicrobial resistant genes for: MRSA (*mecA/c* and *MREJ*), for ESBL (*CTX-M*) and for Carbapenemases (*KPC*, *NDM*, *OXA-48-like*, *VIM* and *IMP*).

This variable is a dichotomous nominal qualitative variable, defined as 'yes' if resistant gens are found or 'no' if they are not.

#### - Secondary outcomes:

- **Complications:** Events or additional conditions that arise as a result of nosocomial pneumonia. These complications can vary in severity and prognosis for the patient and may require additional medical interventions for management and treatment. Additionally, they can extend hospital stays and impact the prognosis of the study's participants.



This variable is a polytomous qualitative variable as it can have different categories, including empyema, abscess and parapneumonic Effusion, as well as bacteremia, others, or no complications. In order to assess these variables and with the aim of not interfere to healthcare professionals, the identification of the aforementioned complications will be based on the diagnostic code assigned at the patient's discharge or death, relying on the *ICD-10-CM/PCS* system code (*International Classification of Diseases, 10<sup>th</sup> revision, clinical revision/ procedure coding system*) used at Santa Caterina Hospital as the standard for coding clinical and healthcare data for the catalog of Diagnoses and Procedures of CatSalut.

- **Length of hospital stay:** Total time patient stays in the hospital, from randomisation to discharge. It will be measured using a quantitative variable in days.
- **30-days hospital readmission:** Need for hospital readmission within 30 days after randomisation will be measured using a dichotomous nominal qualitative variable with the options 'yes' or 'no'.
- **30-days mortality:** Death from any cause up to 30 days after randomisation will be measured using a dichotomous nominal qualitative variable defined as 'yes' or 'no'.

#### **COVARIABLES:**

- **Age:** Older age involves higher severity and mortality. It is a quantitative variable measured in years, but categorised in the following intervals, resulting a polytomous qualitative ordinal variable:
  - < 65 years
  - 65- 75 years
  - 70 – 75 years
  - 75- 80 years
  - 85 – 90 years
  - > 90 years
- **Sex:** The individual's sex as reported in the clinical history will be measured as a dichotomous nominal qualitative variable with male (M) or female (F) as the two options.

- **Risk factors for multi-drug resistant organisms:** Patient's risk factors that increase the likelihood of developing infections caused by MDRO are: septic shock, previous antibiotic treatment, hospital stay > 5 days, acute respiratory distress syndrome, acute kidney failure, immunosuppression, prevalence of > 25% of MDR pathogens in the hospital unit or previous colonisation by MDR pathogens.

This variable will be measured using a dichotomous nominal qualitative variable defined as 'yes' or 'no'. The information to complete this covariate will be collected from the clinical history.

- **Comorbidities:** Immunocompromised and chronically ill patients have a higher risk of nosocomial infections. Main comorbidities are categorised into as follows: Chronic Obstructive Pulmonary Disease (COPD), High Blood Pressure (HBP), Body Mass Index (BMI) > 30, chronic kidney disease (CKD), others, or no comorbidity.

This covariate is a polytomous qualitative variable as it can have different categories. BMI is calculated according to the following formula:  $BMI = Weight/height^2$  and expressed in  $Kg/m^2$ . The rest of the items will be collected from the clinical history.

- **Known allergy to antibiotics:** When selecting a treatment for HAP it is important to consider any known allergy to antibiotic, especially penicillin.

This covariate is measured using a dichotomous nominal qualitative variable defined as 'yes' or 'no'. To determine if the patient is allergic to an antibiotic or not, it is necessary to review their clinical history for positive allergy tests or severe adverse reactions directly related to the drug. If the patient experiences an adverse relation during the treatment of nosocomial pneumonia, it will be considered an outlier and will not be taken into account when analysing the data.

**Table 7:** Description of the variables included in the study

|                            | VARIABLE  | TYPE                           | CATEGORY OF VALUES  |
|----------------------------|---|--------------------------------|---|
| <b>STUDY VARIABLE</b>      | Implementation of Film Array Pneumonia Plus Panel | Dichotomic nominal qualitative | Yes /No   |
| <b>DEPENDENT VARIABLES</b> | <b>MAIN OUTCOME</b>                               |                                |   |
|                            | Acquisition of MDRO                               | Dichotomic nominal qualitative | Yes / No  |
|                            | <b>SECONDARY OUTCOME</b>                          |                                |   |
|                            | Complications                                     | Polytomous qualitative         | Empyema / Abscess / Parapneumonic effusion/ Bacteriemia / Others / No complications                   |
|                            | Length of hospital stay                           | Quantitative                   | Numerical (days)  |
|                            | 30-days hospital readmission                      | Dichotomic qualitative         | Yes / No  |
|                            | 30-days mortality                                 | Dichotomic qualitative         | Yes / No  |
| <b>COVARIABLES</b>         | Age   | Polytomous qualitative ordinal | < 65 years / 65 -70 years / 70 – 75 years / 75 - 80 years / 80- 85 years / 85 – 90 years / > 90 years |
|                            | Sex   | Dichotomic nominal qualitative | Male (M) / Female (F)   |
|                            | Risk factors for MDRO                             | Dichotomic nominal qualitative | Yes / No  |
|                            | Comorbidities                                     | Polytomous qualitative         | COPD/ HBP / CKD / BMI >30 / Immunosuppression/ Others / No comorbidity                                |
|                            | Allergic to an antibiotic                         | Dichotomic nominal qualitative | Yes / No  |

## 6.5 Study intervention

It has to be mentioned that this before-after study will not randomise all participants.

### **Period before intervention**

In the initial phase of the study, 37 subjects diagnosed with nosocomial pneumonia will be recruited. The established procedure before initiating this project will be followed. Each patient has to undergo a blood test and CXR to confirm the clinical diagnostic suspicion of pneumonia. Following, a nursing professional will collect a sputum sample from the patient to analyse the aetiological diagnosis of pneumonia.

Conventional culture, considered the gold standard technique, will be performed in this first phase. Only good quality sputum samples (less than 10 squamous cells and more than 25 leukocytes per low-power field by Gram Stains) will be accepted and processed for analysis.

The culture results will take approximately five days to obtain. In the meantime, the physician will treat the patient empirically according to the hospital protocol recommendations. For patients with a low prevalence of MDRO, monotherapy with a broad-spectrum antibiotic with antipseudomonal activity will be initiated. In case of patients with risk factors for MDRO, combined treatment will be commenced. Finally, patients with risk factors for MRSA, should also be prescribed Linezolid or Vancomycin, and if ESBL is suspected, a Carbapenem.

The use of immediate targeted antimicrobial treatment, modification or de-escalation to a narrower spectrum antimicrobial regimen is recommended if a sensitive pathogen has been identified. Finally, the duration of antimicrobial therapy will be determined by physicians, following recommendations based on recent guidelines that suggest a duration of 7-8 days.

In addition, the FilmArray technique will be performed to analyse antibiotic resistance in the selected individuals before initiating the intervention. However, healthcare professionals will not be informed of the results obtained.

### **Period after intervention**

In this phase of the study 111 patients diagnosed with nosocomial pneumonia will be consecutively recruited, and the intervention being studied will be initiated. The intervention involves implementing the FilmArray Pneumonia *Plus* Panel, a comprehensive molecular diagnostic test used to detect respiratory pathogens from nasopharyngeal or sputum samples, depending on patient's availability.

Semi-quantitative detection will be performed using real time PCR of 15 bacteria (11 gram-negative and 4 gram-positive) that are known to cause pneumonia. In addition, qualitative detection of 3 atypical bacteria, 9 respiratory viruses and 7 antibiotic resistance markers will be performed. In this phase of the study, antibiotic resistances will be measured using the same method as in the previous phase, that is, directly analysing the samples with the FilmArray.

It is important to note that a notable limitation of the PCR-based test is the challenge of determining whether the pathogen detected is a coloniser or not. Therefore, to address this, a semi-quantification of relative bacterial abundance will also be performed.

The microbiological results will be analysed and interpreted by an expert microbiologist that collaborates with the research team. The expert will take into account the isolated microorganism and the clinical context of each patient. The treating physicians will receive this information within an hour, either through the electronic patient file or by telephone notification. A targeted antibiotic treatment will be initiated in these patients to combat the identified pathogen from the molecular test obtained.

### **6.6 Safety**

The FilmArray is a diagnostic method recently approved by the FDA (Food and Drug Administration), the agency of the United States Department of Health and Human Services. Considering that the study only focuses on changing the method of microbiological analysis of respiratory samples obtained through sputum and does not directly affect the therapeutic management of patients, the riskS associated with the use of this diagnostic technic are non-existent.

The only consideration that needs to be made are those associated with the use of antimicrobial drugs. However, it is important to note that all these drugs have already been approved and are routinely used in the hospital's clinical practice. Adverse events related to the prescribed antibiotics will be collected and communicated to the appropriate authority.

### 6.7 Data collection

Demographic and clinical data of interest will be collected from participants by completing the case report form (**ANNEX 3**). The data will be obtained through a clinical interview with the patient or by accessing in their medical history. To preserve participant anonymity as much as possible, specific identification numbers will be used instead of names and personal data.

After obtaining all the necessary information for the project, the research team will create a computer-based database and transfer the data to an Excel spreadsheet. Finally, they will use the R studio program for statistical inference.

### 6.8 Follow-up

All patients will be assessed daily by a member of the study team until discharge or death. A follow-up visit will be arranged for all participating patients 30 days after discharge. For patients who do not attend the follow-up visit, a structured telephone interview will be used to assess outcomes.

## 7. STATISTICAL ANALYSIS

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The statistical analysis will be carried out by the statistical analyst. For all analyses, a 95% confidence interval will be defined, and the results will be considered statistically significant when the p-value is  $\leq 0.05$ .

### 7.1 Descriptive analysis

Qualitative variables, both dependent and covariables, will be summarised as percentages within each group. Length of hospital stay will be summarised using the medians and interquartile ranges within each group, as the distribution of this variable is asymmetrical. Also, in the case of length of hospital stay, Kaplan-Meier curves will be estimated and drawn for each group.

All these analyses will be stratified by the covariate.

### 7.2 Bivariate inference

The difference in the proportions of qualitative dependent variables (multi-drug resistant organisms and complications) between the two groups (before and after intervention) will be analysed using the chi-square test or the Fisher's exact test if the expected number of cases in a cell is less than 5. For length of hospital stay, 30-day hospital readmissions and mortality, the Mann-Whitney's U test will be applied. To test the difference of the Kaplan-Meier curves, the log-rank test will be used.

### 7.3 Multivariate analysis

A dichotomous variable will be calculated for each of the complications (empyema, abscess, parapneumonic effusion, bacteriemia or others). Logistics regressions will be estimated adjusting for covariates to avoid potential confounding by the effect of other variables of the FilmArray implementation on the dependent qualitative variables. In the case of length of hospital stay, we will use a Cox's regression model controlling for all the covariates.

As a measure of association, odds ratio (for logistic regression) and hazard rate (for Cox regression) will be calculated to estimate the risk of the different groups.

## 8. ETHICAL AND LEGAL CONSIDERATIONS

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### Ethical principles:

The study will be conducted in accordance with the requirements established in the **Declaration of Helsinki** of Ethical Principles for Medical Research Involving Human Subjects signed by the World Health Association (last updated in October 2013 during the 64<sup>th</sup> General Assembly held in Fortaleza, Brazil).

In addition, this project will follow the **Principles of Biomedical Ethics from Beauchamp and Childress**, commonly known as the four fundamental ethical principles:

1. **Autonomy:** the patients values and personal choices will be respected throughout the study, 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*”.

An information sheet on the study protocol (**ANNEX 4**) will be provided and explained in plain language to give participants with the necessary knowledge and understanding of the procedure

Investigators must obtain written informed consent (**ANNEX 5**) from each participant before the study begins, to ensure that they understand what they are signing, what it means, that they are free to refuse to participate and that they can withdraw from the study at any time without prejudice. If informed consent cannot be obtained, it will be procured by proxy, and if even that is not possible, it will be obtained as soon as possible afterwards.

Finally, any participant who wishes to withdraw from the project will be excluded from the final sample analysis by signing the Informed Consent Withdrawal Form (**ANNEX 6**) to ensure their freedom of choice and in the knowledge that this will not affect their health care.

2. **Beneficence:** it is the moral obligation to act for the benefit of others. In this study, the inclusion criteria have been described with the intention of including patients with nosocomial pneumonia who will benefit most from the study procedure. However, treatment management will not be inferior for any patient, as they will be treated with the same available antibiotic as the patients not included in the trial, always using evidence-based practices.



3. **Non-maleficence:** there is no malicious intent towards the study participants. Patients who meet exclusion criteria will be excluded from the project as they would not benefit from this study. At all times, clinicians will be trained to use their judgement to override the FilmArray results to choose the best antibiotic treatment when clinically indicated, knowing that doing no harm is paramount in these circumstances.
4. **Justice:** The study will respect an equitable distribution of healthcare resources and avoid any discrimination between patients to ensure the principle of equity. If a patient meets the inclusion criteria and there are no exclusion criteria, they will have the same opportunity to participate in the trial, ensuring fairness and equality between people.

### **Comitè d'Ètica d'Investigació Clínica (CEIC)**

Prior to the start of the research, the present project will be submitted to the Clinical Research Ethics Committee of the Hospital Trueta in Girona for review. The committee will ensure that the protocol complies with the ethical requirements for approval and once approved, the project will begin. The control system which will be implemented during the research will be in compliance with the "*Ley 14/2007, de 3 de julio, de Investigación biomédica*".

### **Data protection and confidentiality:**

The confidentiality of the participants will be protected by anonymising the data collected and assigning them a specific identification number for the study. Only the research team will have access to the data collected. No third party will have access to this information. Therefore, this study guarantees the confidentiality and anonymity of all participants' data, and all data will be used exclusively for the purpose of the study.

Personal data will be protected in accordance with the "*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*".

**Transparency:**

Finally, all researchers involved in this study must declare that they have no conflicts of interest. The research will be conducted in the absence of any commercial or financial relationship that could guarantee that there is no commercial bias. The authors will declare the primary aim of this research is to develop generalisable knowledge to improve human health and the quality of life of patients.

Researchers also agree to publishing all data and results with full transparency including unfavourable data or events. Should there be any deviation from the original project plan during the study, the research team will contact the participants to inform them in full transparency and a new consent form signed by each participant will be required to continue the study.

## 9. WORKING PLAN AND CHRONOGRAM

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### 9.1 Research team members

The study will be conducted by a research team composed by:

- **Research Team (RT):** They will oversee the general coordination, economic management, interpretation of the results of the statistical analysis and the writing of the article and its publication.
  - **Project Coordinator (PC):** It will be the clinical physician Albert Gómez, head of the Department of Internal Medicine at the Santa Caterina Hospital and assistant professor of microbiology at the Faculty of Medicine at the University of Girona. He will direct all phases of the study and collect the necessary data from the hospital.
  - **Main Investigator (MI):** It will be Marçal Navarro, currently a 6<sup>th</sup> grade medical student at the Faculty of Medicine at the University of Girona
  - **Co-Investigator (CI):** It will be the PhD medicine and clinical physician Maite Serrando, physician at the ICS-IAS Girona Clinical Laboratory and assistant professor of laboratory medicine at the University of Girona.
- **Collaborators (CO):**
  - **Internal medicine physicians:** All physicians from internal medicine ward and junior doctors that want to collaborate in the study will participate in the recruitment of new patients for the study and follow-up of patients.
  - **Laboratory personnel:** Laboratory technicians, clinical laboratory physicians and microbiologist who will be responsible for the preparation, processing, and analysis of samples collected for microbiological and molecular diagnostic.
  - **Nursing personnel:** They will be responsible to monitor the patient's vital signs, collecting sputum samples, and also performing necessary analytical extractions.
  - **Radiology staff:** Radiologic technicians and specialist radiologists, responsible for conducting diagnostic imaging techniques (CXR and/or lung ultrasound) to diagnose pneumonia.
- **Professional Statistician (PS):** a qualified statistician from *Institut d'Investigació Biomèdica de Girona (IDIBGI)* will be hired to carry out data obtained and statistical analysis.

## 9.2 Work plan

The estimated duration of the entire study is 60 months, which corresponds to 5 years. Throughout the study period, regular follow-up meetings will be scheduled for all the member of the research team. The steps done in this quasi-experimental study will be carried out in the following order, grouped into 5 stages, each consisting of different activities:

### ○ **Stage 1: Elaboration of protocol and study design**

- *4 months: November 2023 – January 2024.*

1. **First session** (*November 2023, completed*): the study development was planned and agreed by the PC (Dr. Albert Gomez) and the MI (Marçal Navarro). During this meeting, the main objectives, hypothesis and methodology were defined.
2. **Bibliographic research and protocol writing** (*November 2023- January 2024, completed*): Extensive bibliographic research has been carried out to obtain the latest evidence on the implementation of Film Array and to write the protocol.
3. **Recruitment of personnel** (*January 2024, completed*): The PC has proposed all of the aforementioned collaborators to participate in the study. The objectives of the study were then explained, and tasks were assigned.

### ○ **Stage 2: Ethical evaluation**

- *4 months: January – April 2024.*

4. **Ethical evaluation and approval** (*January - April 2024*): Once the protocol has been done, the PC will present it to the CEIC, to propose the study and await its approval. Any suggestions will be considered and modified accordingly.

### ○ **Stage 3: Sample recruitment, intervention and data collection**

- *40 months: May 2024 – September 2027.*

5. **Sample recruitment** (*May 2024 – May 2027*): Patients will be recruited into this study using a non-probabilistic consecutive method. They will have to meet all the inclusion criteria and none of the exclusion criteria, as well as sign the informed consent. The sample selection will take 9 months to obtain the first group of 37 participants who will not receive the intervention being

studied in this research. Subsequently, a second group of 111 participants will be recruited for 27 months to receive the FilmArray intervention.

6. **Intervention** (*January 2025 – May 2027*): Implementation of the FilmArray Pneumonia *Plus* Panel in nosocomial pneumonia patients will begin during this period.
  7. **Follow-up**: (*May 2024 – May 2027*): Internal medicine physicians will conduct daily follow-up while patients are in hospital. Additionally, patients will have a follow-up visit arranged 30 days after discharge.
  8. **Post-intervention data collection** (*June – September 2027*): Data collection and development of an anonymised database. This will be done by the RT.
- **Stage 4: Statistical analysis**
- *3 months: October – December 2027*
9. **Statistical analysis** (*October 2027*): It will be performed by a subcontracted statistician, who will analyse the entire database collected using descriptive, bivariate and multivariate analysis. Posteriorly, he/she will interpret the data obtained.
  10. **Results and conclusions** (*November - December 2027*): the PS will present the results to the whole RT, who will oversee the interpretation of the results, prepare tables and graphs and then publish the discussion and conclusions of the study.
- **Stage 5: Publication and dissemination of results**
- *9 months: January- September 2028.*
11. **Article writing, revision and publication** (*January – February 2028*): The MI will write the final article with the results and conclusions obtained. It will be edited and supervised by an English reviewer and published afterwards.
  12. **Dissemination** (*March – September 2028*): The written study will be published as a journal article and the article will be presented to the *Sociedad Española de Medicina interna (SEMI)*. A national congress will be attended to present the results.

A chronogram of the study work plan is presented in **TABLE 8**.

9.3 Chronogram

Table 8: Chronogram of the study work plan.

| STAGES AND ACTIVITIES  | STAFF  | YEARS |    |      |     |      |      |     |      |      |     |      |      |     |      |      |     |      |  |
|--|--------|-------|----|------|-----|------|------|-----|------|------|-----|------|------|-----|------|------|-----|------|--|
|  |        | 2023  |    | 2024 |     |      | 2025 |     |      | 2026 |     |      | 2027 |     |      | 2028 |     |      |  |
|  |        | 11    | 12 | 1-4  | 5-8 | 9-12 | 1-4  | 5-8 | 9-12 | 1-4  | 5-8 | 9-12 | 1-4  | 5-8 | 9-12 | 1-4  | 5-8 | 9-12 |  |
| <b>Stage 1: Elaboration of protocol and study design</b>             |        |       |    |      |     |      |      |     |      |      |     |      |      |     |      |      |     |      |  |
| 1. First Session   | RT     |       |    |      |     |      |      |     |      |      |     |      |      |     |      |      |     |      |  |
| 2. Bibliographic research and protocol writing                       | RT     |       |    |      |     |      |      |     |      |      |     |      |      |     |      |      |     |      |  |
| 3. Recruitment of personnel  | PC     |       |    |      |     |      |      |     |      |      |     |      |      |     |      |      |     |      |  |
| <b>Stage 2: Ethical evaluation</b>                                   |        |       |    |      |     |      |      |     |      |      |     |      |      |     |      |      |     |      |  |
| 4. Ethical evaluation and approval                                   | CEIC   |       |    |      |     |      |      |     |      |      |     |      |      |     |      |      |     |      |  |
| <b>Stage 3: Sample Recruitment, intervention and data collection</b> |        |       |    |      |     |      |      |     |      |      |     |      |      |     |      |      |     |      |  |
| 5. Sample Recruitment  | RT+ CO |       |    |      |     |      |      |     |      |      |     |      |      |     |      |      |     |      |  |
| 6. Intervention  | RT+ CO |       |    |      |     |      |      |     |      |      |     |      |      |     |      |      |     |      |  |
| 7. Follow-up   | RT+ CO |       |    |      |     |      |      |     |      |      |     |      |      |     |      |      |     |      |  |
| 8. Data collection   | RT     |       |    |      |     |      |      |     |      |      |     |      |      |     |      |      |     |      |  |
| <b>Stage 4: Statistical analysis</b>                                 |        |       |    |      |     |      |      |     |      |      |     |      |      |     |      |      |     |      |  |
| 9. Statistical analysis  | PS     |       |    |      |     |      |      |     |      |      |     |      |      |     |      |      |     |      |  |
| 10. Results and conclusions  | RT     |       |    |      |     |      |      |     |      |      |     |      |      |     |      |      |     |      |  |
| <b>Stage 5: Publication and dissemination of results</b>             |        |       |    |      |     |      |      |     |      |      |     |      |      |     |      |      |     |      |  |
| 11. Article writing, revision and publication                        | RT     |       |    |      |     |      |      |     |      |      |     |      |      |     |      |      |     |      |  |
| 12. Dissemination  | RT     |       |    |      |     |      |      |     |      |      |     |      |      |     |      |      |     |      |  |

\* **Abbreviations:** RT: Research team. PC: Project coordinator. CEIC: Comité d'Ètica d'Investigació Clínica. CO: Collaborators. PS: Professional Statistician. 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 12. December

## 10. BUDGET

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### 10.1 Non-included expenses

- **Staff:** all the personnel in the RT and the CO participating in the study will not be paid extra for their participation in the project as they will perform their duties as part of their job. We try to avoid any economic incentive to participate in the study. It is considered that their motivation to join the study should not be incentivised by any economic cost, as the researchers will be rewarded by the scientific prestige and intellectual gains.
- **Materials available:** The hospital where this study will be conducted already has the necessary materials, so they will not be included in the study budget. The materials include:
  - **FilmArray equipment:** The hospital had already rented the equipment to perform the FilmArray multiplex PCR Pneumonia *Plus* Panel. This equipment costs approximately 17.500 € (including shipping), therefore, it is not necessary to include this cost in the final price.
  - **Conventional culture and equipment preparation:** The hospital already owns the necessary equipment to perform conventional cultures, which are the routine diagnostic method for many pathologies. In addition, for each sputum sample, 4-5 Petri dishes with different culture media are required, at an approximate cost of 50 cents per dish. Considering that we will only need to culture 37 samples, we will use approximately 185 dishes with a total cost of 92.5 €. However, the cost of this technique will not be included in the total cost of the study as it is currently the gold standard and is routinely used in hospital laboratories.
- **Travel and meal allowances:** Team meetings will be held virtually. Therefore, these expenses will not be covered.
- **Insurance:** An insurance policy will not be required as the intervention being performed on patients is minimal and already used in routine clinical practice.

## 10.2 Costs included

- Subcontracted services:
  - **Statistical analysis from IDIBGI:** The subcontracted statistical analysis service, carried out by a statistician, will be paid 60 €/h, with an estimated total of 30 hours of work. The final cost will be approximately 1.800 €.
  
- Material costs:
  - **FilmArray costs:** Each FilmArray determination 145€ if performed non-urgently or 170 € if urgent. These prices include personnel preparation costs. To calculate the total cost of all 148 FilmArray, in a hypothetical scenario where they are all performed non-urgently, the total cost would be 21.460 €.
  - **Printing costs:** An informative document (3 pages), informed consent (1 page) and data collection form (1 page) must be printed for each subject. This makes a total of 5 pages per patient. The printing cost is 0,05 €/page. An estimated sample size of 148 patients is required, with an estimated total cost of 37 €. However, it is possible that more information documents may need to be printed, depending on the centre's requirements.
  
- Divulcation costs:
  - **Publication fees:** It is expected that a journal article presenting the main results will be published. For this reason, a publication fee of 2.000 € is assumed.
  - **Linguistic correction:** before submitting the article to the journal, the work of a linguistic proofreader will be required to avoid errors. The budget for this service is 300 €.
  - **National congress:** In order to disseminate the results, the RT will present the results of the study at a national congress. The inscription of attendance to the national congress of Internal Medicine has an approximate cost of 600 € per participant. In addition, travel, accommodation and diets will be also included at an extra cost of 400€. The final cost will be 3.000 €.

The total cost of the study is summarised in **TABLE 9**.



All expenses incurred by the project will be the responsibility of the research team. However, this protocol will be submitted to several applications and calls for public and private funding in order to be carried out and to cover as many costs as possible. However, private funding from commercial sources, will only be accepted if there is no potential conflict of interest.

**Table 9: Total cost of the study**

|                        | TYPE OF COST                          | UNIT COST             | HOURS OR UNITS             | SUBTOTAL                       |
|------------------------|---------------------------------------|-----------------------|----------------------------|--------------------------------|
| Subcontracted services | <i>Statistical analysis</i>           | 60 € / hour           | 30 hours                   | 1.800 €                        |
|                        | <b>Subtotal: 1.800,00 €</b>           |                       |                            |                                |
| Material costs         | <i>FilmArray Pneumonia Plus Panel</i> | 145 € / panel         | 148                        | 21.460 €                       |
|                        | <i>Printing costs</i>                 | 0,05 € / page         | 5 pages x 148 participants | 37 €                           |
|                        | <b>Subtotal: 21.497,00 €</b>          |                       |                            |                                |
| Divulgence costs       | <i>Article publication fees</i>       | 2.000 € / publication | 1                          | 2.000 €                        |
|                        | <i>Linguistic correction</i>          | 300 € / article       | 1                          | 300 €                          |
|                        | <i>National congress</i>              | 1.000 € / attendant   | 3                          | 3.000 €                        |
|                        | <b>Subtotal: 5.300,00 €</b>           |                       |                            |                                |
|                        |                                       |                       |                            | <b>Total Cost: 28.597,00 €</b> |

## 11. LIMITATIONS AND STRENGTHS

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The present study has several limitations that should be acknowledged and are disclosed as follows:

- **Study Design:**

The main disadvantage of the quasi-experimental design is that randomisation is not used when creating the two groups, nor does it define a control, which limits the study's ability to infer a causal relationship between intervention and outcome. As the groups are not equivalent, and the sampling method is a consecutive non-probabilistic one, it is possible that selection bias may occur if a sample does not resemble the reality of the population, leading to unrepresentative results.

However, these problems will be minimised in the multivariate analysis by adjusting for potential confounding factors described in the literature as covariates, thereby increasing interval validity of the study.

- **Sample:**

As previously mentioned, a non-probabilistic method was used to obtain the sample, which may affect the external validity of the study. There is a risk of loss of subjects due to withdrawal, death or loss to follow-up. To account for this limitation, the sample size has already been calculated taking into account an expected loss rate of 10%. All withdrawals and situations where a patient's follow-up is not possible will be recorded in the study.

Furthermore, although the sample has been calculated considering the incidence of nosocomial pneumonia in the centre where the study is conducted, a large sample size would be needed to increase the statistical power of the study. Furthermore, the research team believes that this study is just the first step of many more studies to come afterwards.

- **Investigator variability:**

It must be considered that due to the collaboration of different professionals during the intervention and patient follow-up, there may be some variability between physicians' evaluations in identifying pneumonia-related complications. However, appropriate adherence to hospital protocols and the fact that this is a single-centre study allow for constant communication among all professionals involved in the study and the research team.

- **Economic expenses:**

This is probably the most significant limitation of the study. Although the indication of FilmArray has a clear clinical relevance, as it allows for rapid and straightforward identification of the causative microorganism of pneumonia to provide targeted treatment while detecting bacterial antibiotic-resistant gens, the high cost of performing this molecular technique may pose a clear limitation in establishing it as the gold standard. Therefore, future studies assessing the cost-effectiveness of this intervention are needed.

These 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 hospital-acquired pneumonia.

In contrast, this study also has **numerous strengths** that demonstrate the usefulness and importance of this research.

Initially, it is important to emphasise the innovation of this study, as in the published literature up to date, most of the articles related to the implementation of the FilmArray Respiratory have been directed in patients admitted to the ICU. Therefore, conducting a study that focuses on HAP, without considering VAP, is crucial to initiate and open the door for future research.

Conventional culture method, which has been considered the gold standard until now, takes approximately five days to provide an aetiological diagnosis of infection. In contrast, this molecular technique reduces the time to obtain results to less than an hour while detecting the most prevalent mechanisms of antibiotic resistance in bacteria without the need for any additional techniques.

Furthermore, it does not require extra experienced personnel as it is routinely used in hospitals for other indications such as gastrointestinal infections, meningitis, or sepsis. Also, no further patient samples are required as the same procedure can be performed by collecting just a sputum sample.

Finally, it is not an additional problem or extra cost for the hospital to target the pneumonia pathogen as these antibiotics are readily available and routinely used in hospital settings and are also less expensive than the broad-spectrum antibiotics used to date.

## 12. CLINICAL AND HEALTHCARE IMPACT

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Hospital-acquired pneumonia is a common nosocomial infection among patients admitted to internal medicine wards and is a leading cause of antibiotic overuse and a significant cause of morbidity and mortality.

However, conventional culture, which is currently the standard method, takes at least five days to identify the responsible pathogen of the pneumonia and its susceptibility to antimicrobial treatment. During this time, empirical antimicrobial treatment is prescribed using broad-spectrum antibiotics.

This counterproductive but generalised practice increases the risk of acquiring multidrug-resistant microorganisms with limited assurance that the causative microorganism is covered, exacerbating the alarming exponential increase in antibiotic resistance.

In recent years, molecular diagnostic methods, such as FilmArray rapid multiplex PCR, have emerged with the aim to reduce the time between the microorganism identification and the determination of antibiotic susceptibilities. This method also detects the most prevalent mechanism of antibiotic resistance, exponentially improving the turnaround time of obtaining such results to approximately one hour.

The implementation of the technique could have a significant impact on patient management by providing an alternative approach to address the challenge of early pathogen identification and detection of associated multidrug resistance. Moreover, it enhances antimicrobial stewardship by allowing physicians to tailor the antibiotic to the specific pathogen, reducing the need to prescribe broad-spectrum empiric treatments and ultimately reducing the overall treatment costs.

In addition to all this, the FilmArray could have a significant impact on reducing antibiotic resistance in hospital settings, which is a crucial step in addressing a global health problem. The WHO has already warned that without action, MDRO will become the leading cause of mortality worldwide by 2050, with an estimated 10 million deaths per year, surpassing those caused by road accidents, cancer or diabetes. For this reason, the research team believes that this project could have significant clinical and public health impact.

Based on the reasons mentioned above and the potential impact of FilmArray, it can be concluded that its routine application in diagnosing HAP should be considered as an adjunctive test to provide rapid identification of the aetiological diagnosis and antibiotic susceptibilities of the responsible organism. This will also have a positive impact on clinical patient outcomes by reducing associated complications, hospital stays, readmissions and mortality rates.

### 13. FEASIBILITY

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- **Budget**

The study budget is reasonable, because the hospital already disposes of the necessary equipment required to perform the intervention, the FilmArray equipment. The main economic expense of the study is the cost of each determination. Additionally, apart from the subcontracted statistical analyst, there is no need to hire additional staff.

- **Procedure**

The collection of sputum samples are carried out by trained nurses familiar with the technique, as it does not involve a different method from the one performed routinely. Furthermore, microbiologic and molecular techniques are conducted by trained laboratory personnel with expertise in the procedure. Additionally, molecular results are always validated by a microbiology associate.

- **Time**

Due to the annual incidence of 50 cases of HAP per year at Santa Caterina Hospital, it is feasible to achieve the required sample size of 148 patients in 3 years, which is not considered to be a very long period.

In summary, based on the information provided, it can be concluded that the implementation of this study is feasible, with a **total cost of 28.597,00 €**.

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## 15. ANNEXES

### ANNEX 1: Murray-Washington's criteria

The Murray and Washington's grading system is used to assess the quality of respiratory samples. This criteria involve the evaluation of the balance between upper respiratory epithelial cells, which indicative oropharyngeal contamination, and polymorphonuclear cells, which indicate an active infectious process.

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

To determine the validity of a sample, it must be of good or optimal quality and need to be representative of the lower respiratory tract. The sample should contain more than 25 polymorphonuclear cells and fewer than 10 epithelial cells per low magnification field, which represents **grade 5**.

The system was developed in 1975 to determine the quality of sputum samples. Its use has been extended and extrapolated to tracheal and bronchial aspirates, but it is not applicable to samples of alveolar origin (bronchoalveolar lavage, telescopic catheter, biopsy). Also, it is not applicable to cases of neutropenia, haemorrhage, or samples with no cells or with the presence of other cell types such as lymphocytes.


ANNEX 2: **Data collection from Santa Caterina Laboratory**

| Base de Dades de mostres respiratòries analitzades al laboratori de l'Hospital de Santa Caterina (11/22 – 12/23) |      |      |          |                     |          |  |                                      |
|--|------|------|----------|---------------------|----------|--|--------------------------------------|
| Número de mostra   | Edat | Sexe | Data     | Prova(s) realitzada | Resultat | Microorganisme trobat  | Sensibilitat/Resistència Antibiòtica |
| Mostra 1   | 64   | M    | 16/11/22 | PCR + Cultiu        | +        | <i>P. aeruginosa</i>   | Sensible                             |
| Mostra 2   | 55   | F    | 18/11/22 | PCR + Cultiu        | +        | <i>Moraxella catarrhalis</i> +<br><i>Virus Respiratori Sincitial</i>                     | Sensible                             |
| Mostra 3   | 64   | M    | 26/11/22 | PCR + Cultiu        | -        | <i>Candida tropicalis</i>  | Sensible                             |
| Mostra 4   | 53   | F    | 08/12/22 | PCR + Cultiu        | -        | <i>Actinomyces odontolyticus</i>   | Resistent                            |
| Mostra 5   | 53   | F    | 09/12/22 | PCR                 | -        | -  | -                                    |
| Mostra 6   | 55   | F    | 17/12/22 | PCR + Cultiu        | +        | <i>Serratia marcescens</i> +<br><i>S. agalactiae</i> +<br><i>Enterovirus / Rinovirus</i> | Resistent                            |
| Mostra 7   | 65   | M    | 20/12/22 | PCR + Cultiu        | +        | <i>P. aeruginosa</i>   | Resistent                            |
| Mostra 8   | 35   | F    | 22/12/22 | PCR                 | -        | -  | -                                    |
| Mostra 9   | 71   | M    | 22/12/22 | PCR                 | +        | <i>S. pyogenes</i>   | -                                    |
| Mostra 10  | 69   | F    | 01/01/23 | PCR                 | -        | -  | -                                    |
| Mostra 11  | 69   | F    | 04/01/23 | PCR                 | -        | -  | -                                    |
| Mostra 12  | 54   | M    | 09/01/23 | PCR + Cultiu        | +        | <i>Virus Influenza A</i>   | Sensible                             |
| Mostra 13  | 73   | F    | 16/01/23 | PCR                 | +        | <i>Virus Influenza A</i>   | -                                    |
| Mostra 14  | 51   | M    | 25/01/23 | PCR                 | +        | <i>S. pneumoniae</i> +<br><i>Virus Influenza A</i>                                       | -                                    |
| Mostra 15  | 61   | F    | 28/01/23 | PCR                 | +        | <i>H. influenzae</i>   | -                                    |
| Mostra 16  | 53   | M    | 29/01/23 | PCR + Cultiu        | +        | <i>P. aeruginosa</i> +<br><i>Klebsiella aerogenes</i>                                    | Resistent                            |
| Mostra 17  | 61   | F    | 02/02/23 | PCR + Cultiu        | +        | <i>S. aureus</i> + <i>E. cloacae</i>   | Resistent (mecA/C)                   |
| Mostra 18  | 53   | F    | 05/02/23 | PCR + Cultiu        | +        | <i>S. aureus</i>   | Sensible                             |
| Mostra 19  | 70   | F    | 11/02/23 | PCR                 | +        | <i>S. agalactiae</i> +<br><i>S. Pneumoniae</i> +<br><i>Virus influenza A + VRS</i>       | -                                    |
| Mostra 20  | 53   | M    | 15/02/23 | PCR                 | -        | -  | -                                    |
| Mostra 21  | 39   | F    | 17/02/23 | PCR + Cultiu        | +        | <i>K. pneumoniae</i> +<br><i>H. influenzae</i> +<br><i>Enterovirus/Rinovirus</i>         | Resistent<br>(CTX-M-BLEE)            |
| Mostra 22  | 66   | M    | 17/02/23 | PCR                 | +        | <i>H. influenzae</i> +<br><i>S.pyogenes</i> +<br><i>Virus Influenza A</i>                | -                                    |
| Mostra 23  | 53   | M    | 23/02/23 | PCR                 | -        | -  | -                                    |
| Mostra 24  | 52   | M    | 01/03/23 | PCR                 | +        | <i>Virus Influenza A</i>   | -                                    |
| Mostra 25  | 54   | F    | 09/03/23 | PCR + Cultiu        | +        | <i>H. influenzae</i> +<br><i>S. agalactiae</i> +<br><i>S. pneumoniae</i>                 | Resistent                            |
| Mostra 26  | 65   | M    | 13/03/23 | PCR                 | +        | <i>H. influenzae</i> +<br><i>S. pneumoniae</i> +<br><i>Virus Influenza A</i>             | -                                    |
| Mostra 27  | 69   | M    | 13/03/23 | PCR                 | -        | -  | -                                    |
| Mostra 28  | 52   | M    | 13/03/23 | PCR + Cultiu        | -        | <i>Enterococcus faecalis</i>   | Sensible                             |
| Mostra 29  | 75   | F    | 16/03/23 | PCR                 | +        | <i>S. Pneumoniae</i> +<br><i>Enterovirus/Rinovirus</i>                                   | -                                    |

IMPLEMENTATION OF THE FILMARRAY PNEUMONIA PANEL IN PATIENTS WITH NOSOCOMIAL PNEUMONIA

|                  |    |   |          |              |   |   |                                    |
|------------------|----|---|----------|--------------|---|---|------------------------------------|
| <b>Mostra 30</b> | 52 | M | 21/03/23 | PCR + Cultiu | - | <i>Enterococcus faecalis</i>  | Sensible                           |
| <b>Mostra 31</b> | 65 | M | 23/03/23 | PCR          | + | <i>S. pneumoniae</i> +<br><i>Virus Influenza A</i> +<br><i>Enterovirus/Rinovirus</i>  | -                                  |
| <b>Mostra 32</b> | 62 | F | 28/03/23 | PCR          | + | <i>S. pneumoniae</i>  | -                                  |
| <b>Mostra 33</b> | 65 | M | 28/03/23 | PCR          | + | <i>Virus Influenza A</i>  | -                                  |
| <b>Mostra 34</b> | 52 | M | 28/03/23 | PCR          | - | -   | -                                  |
| <b>Mostra 35</b> | 79 | F | 13/04/23 | PCR          | + | <i>H. influenzae</i> +<br><i>S. pneumoniae</i> +<br><i>Enterovirus / Rinovirus</i>    | -                                  |
| <b>Mostra 36</b> | 66 | M | 15/04/23 | PCR          | + | <i>Moraxella catarrhalis</i> +<br><i>S. pneumoniae</i>                                | -                                  |
| <b>Mostra 37</b> | 46 | M | 20/04/23 | PCR          | + | <i>Virus Parainfluenza</i>  | -                                  |
| <b>Mostra 38</b> | 68 | M | 28/04/23 | PCR          | + | <i>H. influenzae</i> +<br><i>S. pneumoniae</i> +<br><i>Enterovirus / Rinovirus</i>    | -                                  |
| <b>Mostra 39</b> | 78 | M | 09/05/23 | PCR + Cultiu | + | <i>S. aureus</i>  | Sensible                           |
| <b>Mostra 40</b> | 79 | M | 23/05/23 | PCR          | - | -   | -                                  |
| <b>Mostra 41</b> | 79 | M | 03/06/23 | PCR          | - | -   | -                                  |
| <b>Mostra 42</b> | 48 | F | 04/06/23 | PCR + Cultiu | + | <i>P. aeruginosa</i>  | Resistent                          |
| <b>Mostra 43</b> | 73 | M | 11/06/23 | PCR          | + | <i>Moraxella catarrhalis</i> +<br><i>S. Pneumoniae</i> +<br><i>H. influenzae</i>      | -                                  |
| <b>Mostra 44</b> | 63 | M | 30/06/23 | PCR + Cultiu | + | <i>S. Aureus</i>  | Resistent                          |
| <b>Mostra 45</b> | 63 | M | 31/07/23 | PCR          | - | -   | -                                  |
| <b>Mostra 46</b> | 65 | M | 14/08/23 | PCR          | - | -   | -                                  |
| <b>Mostra 47</b> | 77 | M | 18/08/23 | PCR + Cultiu | + | <i>E. coli</i> + <i>P. aeruginosa</i> +<br><i>K. pneumoniae</i> +<br><i>S. aureus</i> | Resistent                          |
| <b>Mostra 48</b> | 56 | M | 22/08/23 | PCR          | + | <i>S. aureus</i> +<br><i>Virus Parainfluenza</i>                                      | -                                  |
| <b>Mostra 49</b> | 49 | M | 23/08/23 | PCR + Cultiu | + | <i>E. cloacae</i> +<br><i>K. pneumoniae</i>   | Sensible                           |
| <b>Mostra 50</b> | 74 | M | 24/08/23 | PCR          | + | <i>S. pneumoniae</i>  | -                                  |
| <b>Mostra 51</b> | 65 | M | 25/08/23 | PCR          | - | -   | -                                  |
| <b>Mostra 52</b> | 65 | M | 30/08/23 | PCR          | - | -   | -                                  |
| <b>Mostra 53</b> | 41 | M | 30/08/23 | PCR          | - | -   | -                                  |
| <b>Mostra 54</b> | 41 | F | 31/08/23 | PCR          | + | <i>H. influenzae</i>  | -                                  |
| <b>Mostra 55</b> | 56 | M | 05/09/23 | PCR          | + | <i>Virus Parainfluenza</i>  | -                                  |
| <b>Mostra 56</b> | 40 | M | 30/09/23 | PCR + Cultiu | + | <i>H. influenzae</i> +<br><i>K. pneumoniae</i> +<br><i>S. aureus</i> +                | Sensible                           |
| <b>Mostra 57</b> | 56 | M | 10/10/23 | PCR + Cultiu | + | <i>P. aeruginosa</i>  | Resistent<br>(VIM –Carbapenemases) |
| <b>Mostra 58</b> | 55 | M | 19/10/23 | PCR + Cultiu | + | <i>Enterovirus / Rinovirus</i>  | Resistent                          |
| <b>Mostra 59</b> | 33 | M | 23/10/23 | PCR          | + | <i>S. aureus</i>  | -                                  |
| <b>Mostra 60</b> | 45 | F | 30/10/23 | PCR + Cultiu | - | <i>Enterococcus faecalis</i>  | Sensible                           |
| <b>Mostra 61</b> | 41 | M | 15/11/23 | PCR          | + | <i>S. pneumoniae</i> +<br><i>Enterovirus / Rinovirus</i>                              | -                                  |
| <b>Mostra 62</b> | 45 | M | 16/11/23 | PCR + Cultiu | + | <i>K. pneumoniae</i> +<br><i>S. aureus</i>  | Resistent<br>(CTX – M- BLEE)       |

ANNEX 3: **Case report form**

| DOCUMENT RECOLLIDA DADES DEL PACIENT          |   |   |
|---|---|---|
| <b>Títol de l'estudi</b>                      | Implementació del FilmArray Pneumònia Plus Panel amb pacients amb pneumònia nosocomial.   | <b>DATA:</b><br>___/___/___   |
| <b>ID pacient</b>                             | _____   |  |
| <b>Data de naixement</b>                      | ___/___/___   |   |
| <b>Sexe</b>                                   | <input type="checkbox"/> Femení   | <input type="checkbox"/> Masculí  |
| <b>Comorbidityats</b>                         | <input type="checkbox"/> MPOC <input type="checkbox"/> HTA <input type="checkbox"/> IMC > 30 <input type="checkbox"/> IRC<br><input type="checkbox"/> Immunodeprimit <input type="checkbox"/> Altres (Quina?) <input type="checkbox"/> Cap                      |   |
| <b>Tècnica Diagnòstica</b>                    | <input type="checkbox"/> Cultiu convencional  | <input type="checkbox"/> FilmArray Pneumonia Plus Panel                             |
| <b>Agent etiològic identificat</b>            |   |   |
| <b>Factors de risc per MDRO</b>               | <input type="checkbox"/> SI   | <input type="checkbox"/> NO   |
| <b>Adquisició de MDRO</b>                     | <input type="checkbox"/> SI (Especificar quin)  | <input type="checkbox"/> NO   |
| <b>Al·lèrgia a algun antibiòtic</b>           | <input type="checkbox"/> SI (Especificar quin)  | <input type="checkbox"/> NO   |
| <b>Complicacions associades a la infecció</b> | <input type="checkbox"/> SI (Especificar quines)<br><input type="checkbox"/> Empiema<br><input type="checkbox"/> Abscés pulmonar<br><input type="checkbox"/> Vessament parapneumònic<br><input type="checkbox"/> Bacterièmia<br><input type="checkbox"/> Altres | <input type="checkbox"/> NO   |
| <b>Dies d'estada a l'hospital</b>             | ___ Dies  |   |
| <b>Readmissió hospital als 30 dies</b>        | <input type="checkbox"/> SI   | <input type="checkbox"/> NO   |
| <b>Mortalitat als 30 dies</b>                 | <input type="checkbox"/> SI   | <input type="checkbox"/> NO   |

**ANNEX 4: Informative Document**

| <b>FULL D'INFORMACIÓ AL PACIENT</b> |  |
|-------------------------------------|--|
| <b>TÍTOL DE L'ESTUDI</b>            | Implementació del FilmArray Pneumònia Panel amb pacients amb pneumònia nosocomial. |
| <b>CENTRE ASSISTENCIAL</b>          | Hospital Santa Caterina  |
| <b>INVESTIGADOR PRINCIPAL</b>       | Marçal Navarro Pera  |

**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 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), per tal d'identificar de forma més ràpida el microorganisme causant de la infecció i així poder ajudar a guiar l'elecció del tractament antimicrobià.



El que volem aconseguir és veure si l'aplicació d'aquesta tècnica ens permet a llarg termini disminuir les resistències bacterianes als antibiòtics a nivell hospitalari. A curt termini, observar si hi ha una millora en l'evolució clínica del pacient, les complicacions associades, la disminució del temps d'estada hospitalària, les readmissions hospitalàries i la mortalitat.

### ***Descripció de l'estudi***

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L'estudi inclourà un total de 148 pacients majors de 18 anys ingressats a l'hospital de Santa Caterina i diagnosticats de pneumònia nosocomial, aquella que es presenta després de 48 hores des de l'ingrés a l'hospital, i que no sembla estar-se incubant en el moment de l'ingrés.

Per tal de poder avaluar els objectius desitjats, hi haurà un primer grup de 37 pacients als quals no se'ls aplicarà l'eina de diagnòstic molecular que estem estudiant i s'analitzarà la mostra obtinguda amb la tècnica diagnòstica de rutina actual, el cultiu convencional.

Una vegada s'hagi aconseguit aquests 37 primers pacients, s'iniciarà la implementació del FilmArray i es reclutaran 111 pacients, als quals s'analitzarà la mostra obtinguda a través de l'esput del pacient amb la tècnica diagnòstica que estem estudiant, el FilmArray.

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.

### ***Risc i beneficis***

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El FilmArray és un mètode diagnòstic de recent aparició que ha estat aprovat per la FDA, 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 riscos 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***

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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 garantía 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 personas físicas en lo que respecta 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***

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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.

**ANNEX 5: Informed Consent Form**

| <b>FULL DE CONSENTIMENT INFORMAT</b> |  |
|--------------------------------------|--|
| <b>TÍTOL DE L'ESTUDI</b>             | Implementació del FilmArray Pneumònia Panel amb pacients amb pneumònia nosocomial. |
| <b>CENTRE ASSISTENCIAL</b>           | Hospital Santa Caterina  |
| <b>INVESTIGADOR PRINCIPAL</b>        | Marçal Navarro Pera  |

Jo \_\_\_\_\_, amb DNI \_\_\_\_\_, de nacionalitat \_\_\_\_\_, major d'edat i amb domicili a \_\_\_\_\_.

Afirmo que:

- He rebut i llegit el Full d'Informació sobre l'estudi que se m'ha entregat.
- He rebut la informació suficient sobre les característiques i objectius de l'estudi, els possibles riscos i la importància de la meua contribució per l'avanç mèdic.
- He pogut fer les preguntes necessàries i desitjades al respecte de l'estudi i han estat respostes de forma satisfactòria.
- He estat informat/ada pel l'investigador \_\_\_\_\_ de les meves implicacions i finalitats de l'estudi.
- Entenc que la meua participació és voluntària.
- Entenc que puc revocar el meu consentiment informat sobre la participació a l'estudi, sense necessitat d'especificar-ne el motiu i sense que això afecti a la meua assistència sanitària.
- Dono permís perquè les meves dades i la meua història clínica siguin utilitzades per l'equip investigador per fins relacionats amb aquest estudi. He estat informat/ada sobre l'ús de caire científic que es farà de les meves dades personals.
- Entenc que les dades facilitades per mi seran totalment confidencials i que puc sol·licitar la retirada i eliminació de les meves dades personals en qualsevol moment de l'estudi.
- Presto la meua 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 <b>PARTICIPANT</b> : | Firma de l' <b>INVESTIGADOR/A</b> : |
| DATA ____ / ____ / ____        | DATA ____ / ____ / ____             |

**ANNEX 6: *Withdrawal of Informed Consent***

| <b>DOCUMENT DE REVOCACIÓ DEL CONSENTIMENT INFORMAT</b> |  |
|--|--|
| <b>TÍTOL DE L'ESTUDI</b>                               | Implementació del FilmArray Pneumònia Panel amb pacients amb pneumònia nosocomial. |
| <b>CENTRE ASSISTENCIAL</b>                             | Hospital Santa Caterina  |
| <b>INVESTIGADOR PRINCIPAL</b>                          | Marçal Navarro Pera  |

Jo \_\_\_\_\_, amb DNI \_\_\_\_\_, de nacionalitat \_\_\_\_\_, revoco el consentiment informat prèviament firmat per participar en l'estudi, sabent que això no repercutirà en la meva atenció sanitària.

Firma del **PARTICIPANT**:

DATA \_\_\_\_ / \_\_\_\_ / \_\_\_\_\_

Firma de l'**INVESTIGADOR/A**:

DATA \_\_\_\_ / \_\_\_\_ / \_\_\_\_\_