PROJECT TITLE: Combined effectiveness of antibiotics and bacteriophages for personalised treatment of ventilator-associated pneumonia caused by Pseudomonas aeruginosa

DURATION: 3 years **PROJECT TYPE:** Unitarian **POPULATION TYPE:** Adult

RESEARCH TYPE: ☑ Basic ☑ Clinical ☐ Epidemiology and health care services

THEMATIC RESEARCH:

Theme	*SubTheme*
Respiratory Infections	Pneumonia and other acute respiratory infections
[Other]: Novel therapeutic approaches	[Other]: Bacteriophages as coadjuvant treatment

DATA FROM THE PRINCIPAL INVESTIGATOR AND ORGANISATION

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AGE: Between 45 and 64 years old

DATA FROM THE REQUESTED FINANCIAL SUPPORT (IN EURO)

Chapter	1st year	2nd year	3rd year	Total
Staff	13.697	14.108	14.531	42.336
Durable equipment				0
Consumables	5.000	20.290	20.290	45.580
Travels		1.500	1.500	3.000
Cost per patient (if applicable)				0
Subcontracting	5.250	4.250	4.004	13.504
Shipment				0
Publications		1.500	1.500	3.000
Patents				0
Other expenses	2.500	25.040	25.040	52.580
Subtotal	26.447	66.688	66.865	160.000
Overheads	6.611	16.672	16.716	39.999
Total	33.058	83.360	83.581	199.999
Evaluation Process Cost*				

*To be filled by the La Marató de TV3 Foundation

TITLE: Combined effectiveness of antibiotics and bacteriophages for personalised treatment of ventilator-associated pneumonia caused by Pseudomonas aeruginosa

KEY WORDS: Bacteriophages [B04.123]; Pneumonia, Ventilator-Associated [C01.748.610.300.500]; Pseudomonas aeruginosa [B03.440.400.425.625.625.100]; Biofilms [G06.120] ; beta Lactam Antibiotics [D27.505.954.122.08

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CENTRE: Fundació de Recerca Clinic Barcelona - Institut d'Investigacions Biomèdiques August Pi i Sunyer

STRUCTURED ABSTRACT:

Background: The compassionate use of bacteriophage cocktails as an adjunct to antibiotics has shown 69% clinical improvement and 48% pathogen eradication in lower respiratory infections. This approach may be superior to antibiotics alone, particularly for ventilator-associated pneumonia (VAP) caused by carbapenem-resistant P. aeruginosa.
Objectives: This study aims to evaluate the safety and efficacy of a novel phage cocktail, alone and in combination with ceftolozane/tazobactam (C/T), for treating VAP caused by carbapenem-resistant P. aeruginosa. Secondary objectives include assessing resistance evolution, respiratory microbiota impact, and biofilm characteristics in endotracheal tubes.
Methods: A new phage library (n=20) will be characterized based on morphology, genomics, lytic activity, and host range against P. aeruginosa clinical strains (n=100). Optimized phage cocktails (n=12) will be tested in vitro, and endotoxin-free formulations will ensure safety. In a porcine VAP model (n=20), animals will be randomized to receive: Control (NaCl 0.9%), C/T (50 mg/kg /25 mg/kg), Phages Cocktail (4 lytic phages, ~1x10^9 PFU/mL), and CT+Phages. Outcomes include P.aeruginosa and phage counts, histopathology, resistances, biofilm and microbiota analysis, inflammatory markers and transcriptomics.
Expected Results: The CT+phages approach is expected to enhance bacterial eradication, reduce histopathological damage, minimize resistances and maintain microbiota balance, highlighting its potential for clinical application.

TÍTOL: Eficàcia combinada dels antibiòtics i bacteriòfags per al tractament personalitzat de la pneumònia associada a ventilació mecànica causada per Pseudomonas aeruginosa

PARAULES CLAU: Bacteriòfags [B04.123]; Pneumònia associada a ventilació mecànica [C01.748.610.300.500]; Pseudomonas aeruginosa [B03.440.400.425.625.625.100]; Biofilms [G06.120]; Antibiòtics β-lactàmics [D27.505.954.

RESUM ESTRUCTURAT:

Antecedents: L'ús compassiu de còctels de bacteriòfags com a coadjuvant als antibiòtics ha demostrat millora clínica (69%) i eradicació del patògen (48%) en infeccions respiratòries. Aquest enfocament podria ser superior als antibiòtics sols, especialment en la pneumònia associada a la ventilació mecànica (VAP) causada per P. aeruginosa (PA) resistent als carbapenems. Objectius: avaluar la seguretat i eficàcia d'un nou còctel de bacteriòfags, sols o junt amb ceftolozane/tazobactam (C/T), per al tractament de la VAP causada per PA resistent als carbapenems. Objectius secundaris: l'evolució de les resistències, l'impacte en la microbiota i el biofilm dels tubs endotraqueals.
Mètodes: Es caracteritzarà una nova col·lecció de bacteriòfags (n=20) segons morfologia, genòmica, activitat lítica i espectre contra soques clíniques de PA (n=100). S'optimitzaran in vitro còctels de bacteriòfags (n=12) lliures d'endotoxines. El tractactament aleatoritzat dels porcs amb VAP (n=20) inclou: Control (NaCl 0,9%), C/T (50 /25 mg/kg), Còctel de 4 Bacteriòfags lítics (1x10^9 UFP/mL) i C/T+Bacteriòfags. Obtindrem recomptes de PA i fags, la histopatología, les resistències, l'anàlisi dels biofilms i la microbiota, els marcadors inflamatoris i la transcriptòmica.
Resultats esperats: en el grup C/T+bacteriòfags esperem la major eradicació bacteriana, major biodiversitat, menor dany histopatològic i resistències, destacant el seu potencial per a l'aplicació clínica.

LAY SUMMARY

Ventilator-associated pneumonia (VAP) is a severe lung infection affecting patients admitted in an Intensive Care Unit (ICU) who require invasive mechanical ventilation as a respiratory support to maintain them alive. As the most common infection in ICUs, VAP carries a high mortality rate - between 24% and 76% - and imposes a significant burden on healthcare resources. Around 20-36% of ventilated patients develop VAP, often caused by Pseudomonas aeruginosa. This bacterium is known for its resistance to antibiotics and ability to form biofilms, which protect it from antibiotic treatments and immune system leading to poor treatment outcomes and complications.
Our project explores an innovative treatment combining phages (viruses that target bacteria, also named bacteriophages) with antibiotics to combat P. aeruginosa in VAP. We will test this in the lab, followed by trials in a model of mechanically ventilated pigs with VAP by P.aeruginosa mimicking real ICU settings. This approach aims to effectively treat VAP by enhancing P. aeruginosa eradication, hinder resistance to antibiotics and phages, and protect the respiratory microbiota. Using phages combined with antibiotics is anticipated to accelerate infection resolution, enhance bacterial clearance, mitigate antimicrobial resistance development, and preserve microbiota balance, supporting its potential for clinical application. Our goals will provide preclinical useful data needed before implementing this strategy in ICU patients with VAP.

RESUM PER A PÚBLIC NO EXPERT

La pneumònia associada a la ventilació mecànica (VAP) és una infecció pulmonar greu que afecta pacients ingressats en una Unitat de Cures Intensives (UCI) que necessiten ventilació mecànica invasiva com a suport respiratori per mantenir-se amb vida. Com que és la infecció més comuna a les UCIs, la VAP té una taxa de mortalitat elevada – entre el 24% i el 76% – i suposa una càrrega significativa per als recursos sanitaris. Entre el 20% i el 36% dels pacients ventilats desenvolupen VAP, sovint causada per Pseudomonas aeruginosa. Aquest bacteri és conegut per la seva resistència als antibiòtics i la seva capacitat per formar biofilms, que el protegeixen dels tractaments antibiòtics i del sistema immunitari, provocant resultats terapèutics deficients i complicacions.
El nostre projecte explora un tractament innovador que combina fags (virus que ataquen bacteris, també anomenats bacteriòfags) amb antibiòtics per combatre P. aeruginosa en la VAP. Provarem aquesta estratègia al laboratori, seguida d'experiments en un model de porcs ventilats mecànicament amb VAP per P. aeruginosa, imitant condicions reals d'una UCI. Aquest enfocament té com a objectiu tractar eficaçment la VAP, incrementant l'eradicació de P.aeruginosa, frenar la resistència als antibiòtics i als fags, i protegir la microbiota respiratòria. L'ús de fags combinats amb antibiòtics s'espera que acceleri la resolució de la infecció, millori l'eliminació bacteriana, mitigi el desenvolupament de resistències antimicrobianes i preservi l'equilibri de la microbiota, donant suport al seu potencial per a l'aplicació clínica. Els nostres objectius proporcionaran dades preclíniques útils necessàries abans d'implementar aquesta estratègia en pacients d'UCI amb VAP.

1. PRESENTATION OF THE HYPOTHESIS AND DESCRIPTION OF THE PROJECT OBJECTIVES

Phage therapy combined with antibiotics represents a novel, personalized approach to treating P. aeruginosa ventilator-associated pneumonia (VAP). This strategy has the potential to be superior to antibiotic therapy alone, particularly in clinical scenarios involving carbapenem-resistant P. aeruginosa susceptible to ceftolozane/tazobactam (C/T). We hypothesize that this approach will result in higher P. aeruginosa eradication rates, reduced severity of histopathological lesions, a lower risk of resistance development to both phages and antibiotics, and minimal disruption to the host microbiota.

Main objective: Evaluate the safety and efficacy of a phage cocktail, alone and in combination with C/T, with a focus on pathogen clearance using a porcine model of VAP caused by a carbapenem resistant P.aeruginosa. The primary outcome is P. aeruginosa counts in lung tissue, while the severity of histopathological lesions will also be considered.

Secondary objectives:

Co-evolutionary dynamics of resistance: Investigate how P. aeruginosa adapts to both C/T and phages over time, assessing whether the combined treatment not only delays but also disrupts resistance development.
Biofilm-associated infection parameters: Quantify bacterial load, biofilm thickness, and P. aeruginosa viability within endotracheal tube (ETT) biofilms to evaluate the potential benefits of phage-antibiotic therapy in preventing and treating biofilms.
Microbiota biodiversity: Assess the impact of phage therapy and C/T on Bronchoalveolar lavage (BAL), lung, and ETT microbiota diversity and composition, determining whether phage therapy selectively targets P. aeruginosa while preserving native microbiota.
Safety: Evaluate key safety indicators, including adverse events, pharmacokinetics/pharmacodynamics (PK/PD), clinical and laboratory parameters, pathogen burden (quantitative cultures of respiratory secretions), hemodynamic stability, and inflammatory markers. Additionally, perform phage immune neutralization assays and assess tissue impact using spatial transcriptomics.

2. BACKGROUND AND STATE OF THE ART, INCLUDING RELEVANT BIBLIOGRAPHY

Ventilator-associated pneumonia (VAP) is the most prevalent device-associated respiratory infection acquired in the Intensive Care Unit (ICU). Of the 6,699 cases of pneumonia reported in the latest ECDC report (1), 70.8% were associated with intubation. The mean device-adjusted rate per ICU was 12.6 intubation-associated pneumonia episodes per 1,000 intubation-days, ranging from 2.2 in Estonia to 19.1 in France. The most frequently isolated microorganism in VAP is Pseudomonas aeruginosa (20%), and of particular concern is the global increase in carbapenem-resistant strains causing VAP (29.9%) (1, 2). Among the firsts therapeutic options for a VAP caused by a carbapenem resistant P.aeruginosa strain is Ceftolozane/tazobactam (C/T).

Ceftolozane/tazobactam (C/T) is a novel β-lactam/β-lactamase inhibitor combination antimicrobial agent that was approved by the Food and Drug Administration (FDA) in June 2019, based on the results of the ASPECT-NP clinical trial (3), for the treatment of hospital-acquired pneumonia (HAP) and VAP. Ceftolozane is a fifth-generation cephalosporin with significant activity against P. aeruginosa and notable stability against pseudomonal AmpC-mediated resistance. Tazobactam extends the efficacy of this combination by inhibiting many extended-spectrum β-lactamases produced by Enterobacteriaceae.

In a previous study, the authors demonstrated the superiority of C/T over piperacillin/tazobactam against an extensively drug-resistant (XDR) strain of P. aeruginosa (4). Nonetheless, the lung P. aeruginosa burden remained above the desired threshold (>3 CFU/g), pointing to the need for adjunctive or alternative treatment approaches.

Phage therapy is gaining renewed interest as antimicrobial resistance rises, yet its use in human medicine remains limited to countries such as Georgia, Poland, Belgium, France, the United States, and Russia (5-8) . A landmark study (9) demonstrated phage therapy's effectiveness in treating 100 patients with complex infections, including 29 out of 100 lower respiratory tract infections caused by MDR pathogens, of them reporting clinical improvement in 69% (n=20) of cases and eradication of the targeted bacteria in 48% (n=14). Importantly, in a dataset of 100 cases, eradication was 70% less probable when no concomitant antibiotics were used (odds ratio = 0.3; 95% confidence interval = 0.127–0.749), indicating the benefits of phage-antibiotic synergy (PAS), which enhances treatment efficacy. PAS has shown promise in treating respiratory infections such as cystic fibrosis (10, 11). The mechanism is explained by a fitness trade-off following phage selective pressure that promotes mutations to allow bacteria to subvert phage infection at the expense of their fitness, resulting in reduced virulence, resensitisation to antibiotics, and colonization defects (10). A clinical case of particular interest involves a 77-year-old woman who survived a complicated episode of VAP caused by multidrug-resistant P. aeruginosa. She was successfully treated on day 20 of VAP diagnosis with a combination of intravenous (IV) C/T and a phage cocktail (administered IV and nebulized twice a day), which resulted in no detectable P. aeruginosa in respiratory samples after four days of treatment (12).

Genetically engineered bacteria and bacteriophages offer promising strategies to control dysbiosis and respiratory infections (13, 14). Engineering bacteriophages for "One Health," which addresses human, animal, and plant health, was recognized as a top emerging technology by the World Economic Forum in 2023 (15). However, advancements in using bacteriophages for VAP are hindered by inconclusive data. Although the FDA generally classifies bacteriophages as safe, phage therapy is not standard in managing respiratory infections, largely due to regulatory hurdles (5). Key challenges include limited data from clinical trials evaluating bacteriophages efficacy, emergence of resistance to both antibiotics and bacteriophages, lysogenic transformation, and the need to comprehensively understand the impacts on the host microbiota, systemic immune response, and overall physiological tolerance to bacteriophages therapy (6, 7, 16).

This project aims to investigate novel therapeutic approaches for carbapenem-resistant P. aeruginosa in VAP and endotracheal tube (ETT)-associated biofilm. Recent findings elucidate the connection between gut bacteriophage dysbiosis and gut microbiota dysbiosis, emphasizing the important role of bacteriophages in maintaining microbiota homeostasis (17). Therefore, an added benefit of this therapeutic approach is preserving microbiota balance and limiting resistance to antibiotics and bacteriophages. The project is novel, as limited data exist on the safety and efficacy of bacteriophages for VAP. However, early studies show promise, with murine and porcine models demonstrating a 1.5 log CFU/mL reduction in pathogen burden following 21 hours of bacteriophage therapy alone (18, 19). Additionally, studies have shown a good synergistic effect of bacteriophage cocktails and C/T against P. aeruginosa biofilms grown in endotracheal tubes in vitro (20). Our project faces additional challenges, including its extended 72-hour experiment duration and the evaluation of bacteriophage and C/T combinations in a preclinical pig model with VAP caused by a carbapenem-resistant P. aeruginosa strain.

Over 15 controlled studies are currently investigating phage therapy for bacterial infections such as wounds, bacteremia, urinary tract infections, and cystic fibrosis-related lung infections. A pioneering Phase 1/2a trial by TechnoPhage (NCT06370598) is testing a nebulized bacteriophages cocktail for safety in treating P. aeruginosa in VAP patients. Our preclinical model is built on this advanced knowledge, offering a significant step forward in using phage therapy alongside standard treatments to tackle VAP and ETT-associated biofilm while also preventing the emergence of resistance and preserving respiratory microbiota. Advancing this innovative approach is crucial for addressing unmet clinical needs like VAP, improving antimicrobial and phage resistance management, and supporting the 'One Health' framework, fully aligned with the objectives of the grant.

Beyond the State-of-the-Art:

1. Innovative adjunctive therapy: This project combines bacteriophage therapy and C/T to address two critical issues in critically ill VAP patients: rapid infection treatment and prevention of multidrug-resistant (MDR) strains linked to poor outcomes. Bacteriophages will be administered via dual routes: IV and nebulization.
2. Microbiota preservation: Unlike traditional 7- to 14-day antibiotics that disrupt microbiota, this project uses bacteriophages as a targeted solution to preserve microbial diversity. By boosting treatment efficacy and reducing treatment duration, we aim to protect the microbiome and lower the risk of resistance.
3. Unique validated porcine VAP model: This project utilizes a highly specialised porcine model that accurately replicates human ICU conditions, offering a unique opportunity to generate clinically relevant data with enhanced translational potential for critical care applications.

4. Targeting MDR Pathogens: P. aeruginosa resistant to carbapenems is complicating VAP management. This project explores phage-antibiotic synergy and resistance mechanisms to develop alternative treatments, expanding options against resistant infections.
5. Safety assessments: Key safety indicators include adverse event monitoring, pharmacokinetics/pharmacodynamics (PK/PD), clinical and laboratory parameters, pneumonia severity, histopathology, and quantitative cultures (for P. aeruginosa and phages), hemodynamic stability, inflammatory markers, phage immune neutralization assays, and the tissue impact of the new therapeutic approach (using spatial transcriptomics).
6. We will develop a new collection of bacteriophages and phage cocktails targeting carbapenem-resistant P. aeruginosa, a major concern in VAP and ICU-related respiratory infections.

References

1.European Centre for Disease Prevention and Control (ECDC), 2024, Healthcare-associated infections acquired in intensive care units
https://www.ecdc.europa.eu/en/publications-data/healthcare-associated-infections-acquired-intensive-care-units-annual-0
2.Pace MC et al., 2023, World Journal of Clinical Cases
https://doi.org/10.12998/wjcc.v11.i10.2874
3.Kollef MH et al., 2022, Critical Care (London)
https://doi.org/10.1186/s13054-022-04276-7
4.Motos A et al., 2021, Antimicrobial Agents and Chemotherapy
https://doi.org/10.1128/AAC.00123-21
5.U.S. Department of Health and Human Services, Food and Drug Administration, 2006, Federal Register
https://www.federalregister.gov/documents/2006/08/18/E6-13621/food-additives-permitted-for-direct-addition-to-food-for-human-consumption-bacteriophage-preparation
6.Mitropoulou G et al., 2022, European Respiratory Review
https://doi.org/10.1183/16000617.0112-2022
7.Principi N et al., 2019, Frontiers in Pharmacology
https://doi.org/10.3389/fphar.2019.00513
8.World Medical Association, 2024, WMA Declaration of Helsinki
https://www.wma.net/policies-post/wma-declaration-of-helsinki/
9.Pirnay JP et al., 2024, Nature Microbiology
https://doi.org/10.1038/s41564-024-01567-1
10.Mangalea MR et al., 2020, Infection and Immunity
https://doi.org/10.1128/IAI.00814-20
11.Tagliaferri TL et al., 2019, Frontiers in Cellular and Infection Microbiology
https://doi.org/10.3389/fcimb.2019.00022
12.Maddocks S et al., 2019, American Journal of Respiratory and Critical Care Medicine
https://doi.org/10.1164/rccm.201902-0376LE
13.Moda-Silva LS et al., 2022, Future Microbiology
https://doi.org/10.2217/fmb-2022-0034
14. Mazzolini R et al., 2023, Nature Biotechnology
https://doi.org/10.1038/s41587-023-01987-4
15.World Economic Forum, 2023, Forum Agenda
https://www.weforum.org/reports/top-10-emerging-technologies-of-2023/
16.Jault P et al., 2019, The Lancet Infectious Diseases
https://doi.org/10.1016/S1473-3099(18)30482-1
17.Ishizaka A et al., 2025, Microbiology Spectrum
https://doi.org/10.1128/Spectrum.00998-24
18.Guillon A et al., 2021, British Journal of Pharmacology
https://doi.org/10.1111/bph.15690
19.Prazak J et al., 2019, American Journal of Respiratory and Critical Care Medicine
https://doi.org/10.1164/rccm.201902-0377OC
20.Oliveira VC et al., 2024, Emerging Microbes & Infections
https://doi.org/10.1080/22221751.2024.2420737

3. PRELIMINARY RESULTS

Our research group has pioneered an advanced porcine model for pneumonia research that closely replicates human respiratory infections in ICU settings. Utilizing state-of-the-art clinical equipment and continuous monitoring, this model enables the evaluation of novel therapeutic strategies and disease mechanisms under realistic conditions, including mechanical ventilation and ventilator-associated pneumonia (VAP). This model allows for the recreation of ICU-acquired pneumonia, a persistent healthcare burden, as highlighted in the latest European Centre for Disease Prevention and Control (ECDC) surveillance report on hospital-acquired infections. Our established collaborations with leading pharmaceutical companies, including Pfizer, Bayer, and MSD, have successfully leveraged our porcine model for preclinical safety, efficacy, and preventive studies in ICU-related infections. These partnerships underscore our group's capacity to translate innovative research into clinically relevant therapeutic solutions.

Our preliminary studies point to the need for therapeutic approaches that not only target infections but also preserve or restore the respiratory microbiome. In a mechanically ventilated porcine model challenged with multidrug-resistant Pseudomonas aeruginosa pneumonia, resistant to piperacillin/tazobactam (MIC 64/4 mg/L) and at the upper limit of the ceftolozane/tazobactam (C/T) susceptibility range (MIC 4/4 mg/L), we observed that appropriate initial treatment with C/T effectively reduces bacterial burden, delays resistance development, and mitigates systemic inflammation. However, persistent P. aeruginosa in lung tissue following 48h treatment (4.04 [3.64–4.51] log CFU/g) highlights the need for adjunctive strategies to enhance bacterial clearance and prevent recurrence.

We have also evaluated novel biotherapeutics utilizing attenuated and genetically engineered bacteria as carriers to deliver anti-pseudomonal compounds (hydrolase, alginase, and pyocins) directly to the infection site, targeting P. aeruginosa biofilms in the lungs and endotracheal tubes (ETT). Our results demonstrate that this approach significantly reduces P. aeruginosa burden in respiratory secretions and lung tissue (1-2 Log reduction compared to untreated group, respectively), while also affecting ETT-biofilm thickness (2.17 [1.53–2.50] microns, the lowest among treatment groups, $p = 0.001$). In particular, nebulized administration of this biotherapeutic strategy yielded the lowest ETT-biofilm P.aeruginosa load (0.6 Log reduction compared to other groups). Furthermore, nebulized delivery, compared to intralobar administration, was associated with a significantly higher Shannon diversity index in ETT-Biofilm (4.35 [3.45–4.91] vs. 3.00 [2.33–4.03], $p = 0.039$), suggesting not only pathogenic biofilm reduction but also microbiota restoration. However, the effect of biotherapeutics as an adjunct to conventional antibiotics has not yet been assessed; therefore, their combined use may optimize treatment efficacy.

Building on this, we recently tested bacteriophages against P. aeruginosa ETT-biofilms grown in vitro. Our findings indicate a strong synergistic effect between two tested phages and ceftolozane/tazobactam (C/T). After 72 hours of exposure, the combination of a two-lytic-phage cocktail with C/T resulted in a six-log reduction in P. aeruginosa burden compared to untreated biofilms, and a 1.77-log reduction compared to C/T alone.

Regarding administration routes, preliminary results comparing intravenous (IV) meropenem with combined IV meropenem and nebulized Amikacin revealed a significant 1.5 Log reduction in P. aeruginosa counts in tracheal aspirates, though no significant reduction was observed in lung tissue. These findings, consistent with those from randomized clinical trials, suggest that nebulized antibiotic particles may have limited penetration into the lower airways. Therefore, combining nebulized and systemic treatments could be essential, with IV administration ensuring rapid systemic distribution, while nebulized delivery enhances local antimicrobial activity, especially against biofilm-forming P. aeruginosa. Moreover, combining phage-antibiotic therapy is expected to help suppress the emergence of resistance. Previous studies have shown that inhaled amikacin, when used as an adjunct to IV meropenem, prevents the emergence of meropenem-resistant subpopulations, compared to IV meropenem alone.

4. METHODOLOGY

This 36-month project is structured in 6 working packages (WP). The preclinical study (WP3) includes the sample size calculation as required.

WP1: Expand the bacteriophage collection

Task 1.1: Isolate and characterize new lytic bacteriophages
Using wastewater from diverse sources, we will amplify new lytic bacteriophages targeting Pseudomonas aeruginosa. Our focus will be on a P. aeruginosa strain resistant to carbapenems, with a minimum inhibitory concentration (MIC) of ceftolozane-tazobactam (C/T) at 4/4 µg/mL. This is particularly relevant as carbapenem- resistant P. aeruginosa is a major concern in ventilator-associated pneumonia (VAP). Our approach simulates a clinical scenario in which an effective adjunctive treatment with phage cocktails and C/T could have a significant clinical impact, given that the MIC for C/T falls within the EUCAST susceptibility breakpoint limits.
The morphology and genomic content of the phages will be further characterized via sequencing to determine their phylogenetic relationships. Additionally, we will evaluate the host range and lytic activity of the amplified bacteriophages against a collection of 100 clinical P. aeruginosa strains isolated from VAP patients (registry no. R190311-203) to assess their efficacy against strains with varying antibiotic susceptibility patterns. The bacteriophages exhibiting the broadest host range and greatest phylogenetic diversity will be selected to formulate 12 bacteriophage cocktails each comprising four distinct lytic phages at ~1×10^9 Plaque Forming Units (PFU)/mL. Those demonstrating the most promising potential for phage therapy against clinical P. aeruginosa strains will be integrated into Networks of Bacteriophages such as FAGOMA.

Task 1.2: In vitro evaluation of the effect of bacteriophage cocktails on resensitizing P. aeruginosa biofilms
A 48-hour mature P. aeruginosa biofilm will be grown in vitro on endotracheal tube (ETT) specimens. Three different P. aeruginosa strains resistant to carbapenems and with borderline susceptibility to C/T will be used, aligning with the project's objectives.
The in vitro biofilms will be treated under the following conditions: no treatment, 12 different phage cocktails (each containing four lytic phages at ~1×10^9 PFU/mL), C/T alone (5 µg/mL), or a combination of the phage cocktail and C/T. Treatments will be applied at various time points (12, 24, 36, 48, 60, and 72 hours) following published methods (20). After treatment exposure, the remaining biofilm will be collected for further analysis. Colony-forming unit (CFU/mL) and plaque-forming unit (PFU/mL) counts will be recorded post-treatment for all P. aeruginosa strains, treatment arms, and phage cocktails. Additionally, any changes in antimicrobial susceptibility to phages, carbapenems, and C/T will be assessed. Sequential alterations in bacterial susceptibility in vitro will help identify the 3 most effective phage cocktails for application in preclinical settings.

WP2: Pre-adaptation of the Bacteriophage's cocktail and formulations

Task 2.1: Phage Pre-adaptation
To enhance the phage cocktail's host range and efficacy by reducing bacteriophage resistance evolution, the 3 most effective phage cocktails will be pre-adaptated as done by others before compassionate administration (9). Four input bacteriophages included in each cocktail, will be combined in equal proportions (1:1:1:1 ratio; 1 × 109 PFU/mL). A mature 48-hour biofilm will be cultivated on the surface of endotracheal tubes (ETTs) using various strains of P. aeruginosa (n=5) that are resistant to carbapenems, susceptible to CT and susceptible to the phages' cocktail. The P. aeruginosa ETT biofilms (n=5) will be individually co-incubated with the phage cocktail at 37 °C overnight to facilitate phage-mediated lysis. Those samples exhibiting the higher lysed suspension from each strain will be harvested, pooled, and filtered to produce a combined lysate. This lysate will then be used to treat fresh P. aeruginosa biofilms over a series of 10 consecutive passages (9). At rounds 1, 5, 10 the pooled phage mixture will be tested both before and after training to assess changes in: Bacteriophage host range and Lysis efficiency. The best cocktail will be prepared for formulation (Task 2.2) and selected for the preclinical study (WP3).

Task 2.2: Phage Cocktail Formulation
The phage cocktail will be prepared according to specific criteria for quality and safety, as described (9), with a strong emphasis on rigorous standards for purity, efficacy, and regulatory compliance before administering phage therapy to patients.
Each phage included in the cocktail will undergo sequencing and analysis to screen for undesired genes associated with antibiotic resistance, virulence, or lysogenic transformation. Additionally, prophage induction will be investigated in the bacterial production host genome. The phage preparation will be purified from endotoxins using commercially available kits. The acceptance criterion for the final phage preparations is an endotoxin limit of 5 EU/kg body mass/h, regardless of the administration route. Finally, the bioburden (total viable aerobic count) of each phage preparation will be determined using a validated membrane filtration method.

WP3: Preclinical Study Using a Phage Cocktail, Alone or Combined with C/T, to Treat VAP Caused by Carbapenem-Resistant P. aeruginosa
(See the figure summarizing WP3 in the attached files)

Sample Size Calculation
We expect a reduction in lung P.aeruginosa counts of 3.00 log in the group treated with the combination of C/T and Bacteriophage cocktail compared to untreated pigs, a reduction of 2.00 log compared to C/T alone and a reduction of 2.5 log compared to bacteriophage cocktail alone. The standard deviation for all groups is expected to be 1.25 log CFU/g. To

achieve a statistical power of 80% and an alpha level of 5%, with an effect size of 0.91 for one-way ANOVA, a sample size of five pigs per group (for a total of four groups: control, C/T, phage cocktail, and C/T + phage cocktail) will be sufficient to detect significant differences in P. aeruginosa lung concentration after each treatment.

Study Design
This is a prospective, randomized study in mechanically ventilated pigs with VAP caused by carbapenem-resistant P. aeruginosa.
Subjects: 20 Large White-Landrace pigs (30–35 kg).

Task 3.1: Ventilator-Associated Pneumonia (VAP) Animal Preparation
Animals will be sedated, intubated, and mechanically ventilated for up to 72 hours. Sedatives and analgesics will be administered as described (4). A femoral artery, jugular vein (with a Swan-Ganz catheter), and bladder (via Foley catheter) will be cannulated. Ventilator settings will be adjusted to maintain normocapnia and normoxia.

Bacterial Challenge
Following surgical preparation, pneumonia will be induced via intrabronchial inoculation of 15 mL of P. aeruginosa (10^7 CFU/mL) resistant to carbapenems but susceptible to ceftolozane/tazobactam (C/T) (MIC: 4 µg/mL) through bronchoscopy into each pulmonary lobe.

Randomization
Twenty-four hours after the bacterial challenge, pigs will be randomized into four groups:
1. Control Group (n=5). Every 8 hours: IV administration of 100 mL of saline (NaCl 0.9%) over 1 hour. Every 12 hours: 4 mL of saline (NaCl 0.9%) by inhalation using a vibrating mesh nebulizer (In-line eFlow Nebulizer System; PARI Respiratory Equipment, Midlothian, VA).
2. Ceftolozane/Tazobactam (C/T) Group (n=5). Every 8 hours: IV administration over 1-hour of 50 mg/kg ceftolozane and 25 mg/kg tazobactam diluted in 100 mL Saline, based on previous humanized dosing (4). Every 12 hours: 4 mL of saline (NaCl 0.9%) by inhalation.
3. Phage Cocktail Group (n=5). Every 12 hours: IV administration of 100 mL (1 mL of phage cocktail containing 4 lytic phages at ~1×10^9 PFU/mL, diluted in 100 mL NaCl 0.9%) over 1 hour. Nebulization of 4 mL of undiluted phage cocktail (4 lytic phages, ~1×10^9 PFU/mL) (9). The rationale for administering the phage cocktail IV and nebulized is explained in the preliminary results section.
4.C/T + Phage Cocktail Group (n=5). Same dosages and administration routes as described above.

Task 3.2: Main measurements in pigs under mechanical ventilation and key safety indicators Respiratory and Hemodynamic measurements
Ventilatory parameters will be assessed every 6h and adjusted to maintain physiological blood gases. Key clinical variables of infection will be continuously monitored. Static elastance, airway resistances, and lung and rib cage measurements will be calculated via the rapid occlusion method. Hemodynamics and gas exchange (arterial and venous blood) will be measured. Blood pressures (central and arterial) will be recorded using disposable pressure transducers (Edwards Lifescience, Irvine, CA). Pulmonary arterial pressure, wedge pressure, and cardiac output (via thermo-dilution) will also be monitored.

Microbiological, Histopathology, and Inflammatory Assessments
P. aeruginosa counts (CFU/mL) and phage counts (PFU/mL): Tracheal aspirates (TA) and blood will be cultured prior to the Pseudomonas aeruginosa challenge and at 12, 24, 36, 48, 60, and 72 hours (autopsy). Bronchoalveolar lavage (BAL) fluid will be cultured at baseline and every 24 hours to minimize the impact of repeated sampling on animal stability. Resistance to C/T and the emergence of phage resistance will be monitored. C/T concentrations for PK/PD studies were published (4). At autopsy, P. aeruginosa pneumonia will be confirmed if the pulmonary burden exceeds 3 log CFU/g in at least three lobes. The histopathology of the lung parenchyma will determine the severity of the lesions (no pneumonia, early pneumonia, intermediate pneumonia, advanced pneumonia and resolution of pneumonia).
For biofilm studies, the endotracheal tube (ETT) will be sliced for P. aeruginosa counts, scanning electron microscopy (SEM), and confocal laser scanning microscopy (CLSM), as reported (14). Additionally, 16S rRNA sequencing will be performed to compare baseline, 24h (pneumonia before treatment), and autopsy samples from BAL, lung, and ETT to evaluate the dynamics of microbial diversity in each treatment group. DNA extraction, library preparation, and sequencing will follow the protocols outlined by manufacturers.
Inflammatory cytokines (IL-1β, IL-6, IL-8, IL-10, TNF-α) will be quantified in serum and BAL fluid using bead-based multiplex assays (Luminex, Millipore Iberica).
In summary, key safety indicators in WP3 include: Adverse Event (AE) monitoring, PK/PD studies, clinical and laboratory parameters, P.aeruginosa infection sequential monitoring and at autopsy (microbiology quantitative cultures for P. aeruginosa and phages and histopathologic lesions), hemodynamic stability, and inflammatory markers. Other key safety indicators are described below in WP4.

WP4: Immune response and tissular impact of bacteriophages
We will evaluate the phage immune neutralization and to assess the tissue heterogeneity, cell subpopulations, and cell-cell interactions, providing an in-depth understanding of gene expression changes, signalling pathways, and potential immunomodulatory effects after phage therapy alone or combined with CT.

Task 4.1: Emergence of neutralizing antibodies, according to previous methods (9).
Whole blood samples will be collected before bacteriophage therapy initiation and every 12 hours. Blood will be allowed to

clot for at least 30 min in a vertical position and then centrifuged in a swinging bucket rotor for 10 min at 2,000 g at room temperature. The serum (0.9 mL of a 1:100 dilution) will be mixed with phage suspensions (0.1 mL of 107PFU/mL), and the phage lytic activity will be determined after 30 minutes incubation at 37ºC. Bacteriophage titter will be evaluated before and after incubation with the animal's serum samples by using the double agar overlay plaque assay. Comparison of pre- and post-incubation lytic activity will allow to determine of the proportion of neutralized bacteriophages. Each serum sample will be tested in triplicate.

Task 4.2: Conduct single-cell gene expression analysis, using single-cell spatial transcriptomics, within lung tissues to understand, how individual immune cells, such as macrophages, neutrophils, and lymphocytes react to the treatments. Lung biopsies will be collected and stored at −80°C. By combining gene expression data with histological imaging, molecular data will be linked with visible tissue architecture, enabling new insights into tissue function, disease pathology, treatment safety, and cellular microenvironments after the different treatments. Safety assessments will include identifying potential off-target effects, unintended immune responses, and inflammatory profiles within the cellular microenvironments. Other key safety indicators have been included in WP3.

WP5: Statistical Analysis

Task 5.1: General Statistical Analysis
A continuous statistical analysis will be conducted to ensure the robustness of results and draw meaningful conclusions. Data will be analyzed to compare differences between study groups using appropriate statistical methods tailored to the type and distribution of variables. Findings will be regularly reviewed and presented in various formats, including scientific congresses, project reports, and peer-reviewed publications.

Task 5.2: Microbiome Profiling and Analysis
Microbiome profiling will involve PCR amplification and sequencing of the 16S rRNA gene using the Illumina MiSeq Sequencing System. Data processing will be carried out using the nf-core/ampliseq pipeline, which includes quality control, trimming, error correction, and taxonomic classification using the Silva 138.1 database. Alpha and beta diversity metrics will be calculated in QIIME2. Additional statistical analyses will be conducted in R.

WP6: see dissemination section.

5. WORK PLAN, BROKEN DOWN BY TASK AND INVESTIGATORS, AND TIMETABLE

WP1: Expand the bacteriophage collection (M1-M6)
Task 1.1: Isolate and characterize new lytic bacteriophages (M1-M3)
Task 1.2: In vitro evaluation of the effect of bacteriophage cocktails on resensitizing P. aeruginosa biofilms (M3-M6)
Team involved: PI, all team and hired staff

WP2: Pre-adaptation of the bacteriophages (M6-M12)
Task 2.1: Bacteriophage pre-adaptation (M6-M10)
Task 2.2: Bacteriophage cocktail formulation (M10-M12).
Team involved: PI, ARISTOS PhD, Molecular biologist and hired staff

WP3: Preclinical study using a bacteriophage cocktail, alone or combined with C/T, to treat VAP caused by carbapenem-resistant P. aeruginosa (M13-M36)
Task 3.1: Ventilator-Associated Pneumonia (VAP) (M13-M27).
Task 3.2: Main measurements and key safety indicators respiratory and hemodynamic measurements (M13-M36)
Team involved: PI, all team and hired staff

WP4: Immune response and tissular impact of bacteriophages (M18-M36)
Task 4.1: Emergence of neutralizing antibodies (M18-M29)
Task 4.2: Single-cell gene expression analysis, using single-cell spatial transcriptomics (M26-M36)
Team involved: PI, all team and hired staff

WP5: Statistical analysis
Task 5.1: General statistical analysis (M7-M36)
Task 5.2: Microbiome profiling and analysis (M19-M33)
Team involved: PI, Emeritus Prof., ARISTOS PhD PhD Bioinformatician, Pharmacist

WP6: Management and Dissemination (M6-M36)
Task 6.1: Economic and scientific periodic reports (M6-36)
Task 6.2: Dissemination activities (M7-36)
Team involved: PI, Emeritus Prof., ARISTOS PhD

6. SEX, GENDER AND OTHER DIVERSITY PERSPECTIVES

In this project aimed at evaluating the efficacy of bacteriophage therapy in ventilator-associated pneumonia (VAP), we acknowledge the importance of integrating a gender perspective into research design, data analysis, and interpretation. While our study utilizes female pigs for the preclinical trials, it is essential to clarify that gender-related variables are not expected to influence the outcomes of this study due to the specific conditions of the animal model.

The decision to use only female pigs (Large-White Landrace, 30-35 kg) for this study is driven by ethical considerations and the procedural advantages of transurethral catheterization. In female pigs, this minimally invasive procedure can be performed without the need for surgery, whereas male pigs require a more invasive surgical approach, which may introduce additional variables unrelated to the research question. This consideration of animal welfare is aligned with current ethical standards in preclinical research.

It is important to highlight that the selected female pigs are prepubertal, meaning they are not yet at an age where hormonal fluctuations or reproductive differences could introduce significant biological variation. These factors, such as hormonal, cardiovascular, and metabolic differences, which could potentially affect disease progression and response to therapy in adult pigs, do not apply to the prepubertal females used in this study. As such, the absence of male pigs in this preclinical study will not result in a bias related to gender differences in the context of the project's aims.

Furthermore, while the team is composed of individuals of diverse gender identities, we ensure that all aspects of this study—ranging from phage formulations and bioengineering to animal handling and molecular analysis—are guided by scientific rigor and are free from gender bias. The inclusion of female, male and non-binary researchers reflects a commitment to creating an inclusive environment where the research is driven by scientific merit and not by gender-based factors.

In summary, despite the exclusive use of female pigs, the design and implementation of this study ensure that gender will not impact the results. The prepubertal stage of the animals minimizes any gender-related biological influences, allowing the focus to remain on the efficacy of bacteriophage therapy for VAP.

7. IMPACT

7.1 Intended Impacts

This project aims to generate significant scientific, medical, and societal impacts by advancing knowledge, informing policy, and improving treatment strategies for ventilator-associated pneumonia (VAP) caused by carbapenem resistant Pseudomonas aeruginosa. Below, we outline the intended contributions of this study:

1. Impact on Knowledge Generation
This study will contribute to expanding scientific understanding in multiple ways:
• It pioneers new lines of research in phage therapy, specifically targeting P. aeruginosa biofilms in VAP, an area with limited prior exploration.
• The study integrates principles of microbiology, molecular biology, and clinical medicine, promoting a multidisciplinary approach to tackling antimicrobial resistance.
• It will develop a new collection of bacteriophages pre-adapted to carbapenem-resistant P. aeruginosa clinical strains, which are a major clinical concern in ICU-acquired respiratory infections.
• By elucidating phage-host interactions, resistance mechanisms, and therapeutic synergies with antibiotics, the project will generate valuable insights applicable beyond VAP to other infectious diseases.
• The translational aspect of this research fosters collaboration between laboratory science and clinical applications, advancing precision medicine.

2. Orientation to Societal Needs
The study addresses the urgent need for alternative antimicrobial therapies, as highlighted by the World Health Organization's warnings about the post-antibiotic era. The project is directly oriented to societal needs by:
• Developing an innovative therapeutic option that may alleviate the burden of antibiotic resistance, particularly in ICU-acquired infections.
• Providing a potential solution for managing multidrug-resistant P. aeruginosa, a critical pathogen in healthcare-associated infections.
• Enhancing healthcare preparedness by offering an alternative to traditional antibiotic regimens, which are becoming less effective due to rising resistance.

3. Benefits for Professionals, Policy Makers, and Decision-Makers
The findings of this study could be instrumental in shaping clinical and regulatory landscapes:
• Healthcare professionals will benefit from evidence-based data supporting phage therapy's integration into clinical practice.
• Policy makers and regulatory bodies, including the European Centre for Disease Prevention and Control, can use this research to inform guidelines on antimicrobial resistance management.
• Pharmaceutical and biotechnology companies may leverage study outcomes for the development and commercialization of phage-based treatments, fostering innovation in antimicrobial therapeutics.
• The research aligns with national and international efforts to address antimicrobial resistance, aiding in policy formulation for novel treatment options.

4. Impact on Health Treatments, Diagnosis, Prevention, and Care
The project has direct implications for medical treatment and healthcare practices:
• The proposed synergistic approach combining phage therapy with ceftolozane/tazobactam may enhance treatment efficacy, potentially reducing treatment duration and the emergence of resistance.
• Rigorous preclinical validation using animal models will pave the way for clinical trials, accelerating the translation of phage therapy into routine medical care.
• Improved treatment strategies for VAP could potentially reduce ICU stays, healthcare costs, and patient mortality associated with antibiotic-resistant infections.
• By preserving native microbiota, phage therapy offers a more targeted approach compared to broad-spectrum antibiotics, minimizing collateral damage to beneficial microorganisms.

5. Impact on Patients and the Public
The societal impact of this research extends to patient care and public health:
• The study aims to provide critically ill patients with a more effective, personalized treatment option, potentially improving recovery rates and overall prognosis.
• A reduction in antibiotic-resistant infections will have long-term benefits for public health, decreasing the spread of resistant strains and reducing dependence on last-resort antibiotics.
• Increased awareness and acceptance of phage therapy among healthcare providers and the general public could lead to broader adoption and demand for such treatments.
• The findings may contribute to patient advocacy efforts, promoting access to innovative therapies in healthcare settings.

6. Additional Academic, Professional, and Societal Impacts
Beyond direct medical applications, this study is expected to generate broader impacts:
• Academic Contributions: The research will lead to high-impact publications, conference presentations, and knowledge dissemination through scientific networks, fostering further investigation into bacteriophage applications.
• Training and Capacity Building: The project will provide learning opportunities for early/mid-career researchers, fostering expertise in translational microbiology and antimicrobial resistance research. Two international fellows (1 awarded with an ARISTOS-MSCA) have currently joined the team to work in this research topic.

• Regulatory Advancements: Spain currently lacks specific regulations for phage therapy; this research may provide critical data to support regulatory advancements, facilitating the implementation of phage-based treatments.
• Economic and Industrial Impact: The development of phage therapy has the potential to stimulate economic growth by creating opportunities for biotech startups and pharmaceutical companies investing in antimicrobial innovations.
• Global Health Impact: By contributing to the global fight against antibiotic resistance, the study aligns with international health priorities and could inform strategies for managing drug-resistant infections worldwide.

Overall, by addressing a pressing medical challenge, this project holds the potential to generate lasting impacts across multiple domains, from advancing scientific knowledge to improving patient outcomes and shaping healthcare policies. Through rigorous research, interdisciplinary collaboration, and translational applications, this study aims to drive meaningful progress in the field of antimicrobial resistance and precision medicine.

7.2 Communication and Dissemination Plan

This work package (WP6) ensures the structured dissemination of the project's progress and findings, facilitating effective communication with scientific, institutional, and public audiences. The objective is to maximize the project's impact through high-quality scientific dissemination, strategic outreach, and institutional engagement, ensuring that key results are effectively communicated to relevant stakeholders.
Task 6.1: Scientific and economic reporting
This task focuses on systematically documenting the scientific and financial execution of the project to ensure transparency, alignment with funding requirements, and efficient communication with key stakeholders.
• Scientific progress reporting: Regular updates will be compiled to document advancements in research, experimental milestones, and key findings.
• Financial execution reports: Expenditure tracking and reporting will be conducted, ensuring accountability and compliance with funding agency requirements. A detailed execution report will be produced for the funding entities as required.

Task 6.2: Dissemination Activities
A dedicated dissemination strategy will be implemented to maximize the project's visibility and outreach. Key activities include:
• Development of a dedicated project website: A professional website will be designed as a central hub for communicating project objectives, research updates, key findings, and publications.
• Production of high-quality science communication materials: Infographics, posters, and other visually engaging materials will be designed to support dissemination efforts across conferences, institutional channels, and digital platforms.
• Video production for project communication: Two professionally produced videos will capture key project milestones and showcase statements from researchers.
• Scientific animation explaining project science: A custom scientific animation will visually communicate the biological mechanisms underlying the phage-antibiotic synergy to both scientific and non-scientific audiences.
• Sponsoring a dedicated session at a scientific event, such as the International Symposium on Infections in Critically Ill Patients (ISICIP), which is held annually in Barcelona: The project will support a roundtable discussion focusing on new therapeutic approaches for VAP.

8. FUNDING STATEMENT

Include funding? ☑

FINANCIAL SUPPORT REQUESTED TO OTHER PUBLIC OR PRIVATE ENTITIES RELATED TO THIS PROJECT

Financing entity	Amount	Concept	Current Status
Spanish Society of Pulmonology and Thoracic Surgery	12000,00	Research grant covering preliminary in vitro studies not included in this Marató project	Executed

9. COSTS SUMMARY IN EURO

CHAPTER: STAFF

Concept	Own contribution	Finantial by other organizations	Requested Support
Part-time laboratory technician	0	0	13.697
Part-time laboratory technician	0	0	14.108
Part-time laboratory technician	0	0	14.531
Subtotal:	0	0	42.336

JUSTIFICATION: Laboratory technician for part-time support

CHAPTER: DURABLE EQUIPMENT

Concept	Own contribution	Finantial by other organizations	Requested Support
Subtotal:			

JUSTIFICATION:

CHAPTER: CONSUMABLES

Concept	Own contribution	Finantial by other organizations	Requested Support
Laboratory consumables (kits and reagents, flow cells, culture media, E-test strips, C/T)	0	0	5.000
Animal Lab Consumables (Animals, drugs/medications (C/T supplemetnary cost: 602 euros/animal for goups receiving C/T, catheters, disposable ICU material) and Laboratory consumables	0	0	20.290
Animal Lab Consumables and Laboratory consumables	0	0	20.290
Subtotal:	0	0	45.580

JUSTIFICATION: Laboratory and Animal Surgical room consumables

CHAPTER: TRAVELS

Concept	Own contribution	Finantial by other organizations	Requested Support
Expenses for travelling associated to dissemination of project results to international congresses	0	0	1.500
Expenses for travelling associated to dissemination of project results to international congresses	0	0	1.500
Subtotal:	0	0	3.000

JUSTIFICATION: International dissemination of project results

CHAPTER: COST PER PATIENT (if applicable)

Concept	Own contribution	Finantial by other organizations	Requested Support
Subtotal:			

JUSTIFICATION:

CHAPTER: PUBLICATIONS

Concept	Own contribution	Finantial by other organizations	Requested Support
Open Access publication costs	0	0	1.500
Open Access publication costs	0	0	1.500
Subtotal:	0	0	3.000

JUSTIFICATION: Costs associated to Open access publications

CHAPTER: SUBCONTRACTING

Concept	Own contribution	Finantial by other organizations	Requested Support
Dissemination and Communication activities (Reporting,Website, infographics, video)	0	0	5.250
Dissemination and Communication activities (Reporting,Website, infographics, video)	0	0	4.250
Dissemination and Communication activities (Reporting,Website, infographics, video)	0	0	4.004
Subtotal:	0	0	13.504

JUSTIFICATION: Services for dissemination and outreach-WP6

CHAPTER: SHIPMENT

Concept	Own contribution	Finantial by other organizations	Requested Support
Subtotal:			

JUSTIFICATION:

CHAPTER: PATENTS

Concept	Own contribution	Finantial by other organizations	Requested Support
Subtotal:			

JUSTIFICATION:

CHAPTER: OTHER EXPENSES

Concept	Own contribution	Finantial by other organizations	Requested Support
Platforms: Animal transport, housing, air supply, genomic and transcriptomic sequencing, histophatology, blood analyses	0	0	2.500
Platforms: Animal transport, housing, air supply, genomic and transcriptomic sequencing, histophatology, blood analyses	0	0	25.040
Platforms: Animal transport, housing, air supply, genomic and transcriptomic sequencing, histophatology, blood analyses	0	0	25.040
Subtotal:	0	0	52.580

JUSTIFICATION: Platforms: Animal Housing, Genomic and trasncripto

10. ACCEPTANCE OF CONDITIONS

☑ I accept the Conditions

11. APPROVAL OF THE COMMISSION'S RESEARCH CENTRE

Documentation attached? ☑ Yes

12. APROVAL OF THE HUMAN SUBJECTS PROTECTION COMMITTE AND ANIMAL EXPERIMENTATION COMMITTEE

12a. REPORT FROM THE CLINICAL RESEARCH ETHICS COMMITTEE OF THE CENTRE.

Documentation attached? ☑ Yes

12b. REPORT FROM THE ANIMAL EXPERIMENTATION COMMITTEE OF THE CENTRE.

Documentation attached? ☑ Yes

13. STATEMENT OF COMPLIANCE WITH GOOD SCIENTIFIC PRACTICE

☑ The organisation I represent has a CODE OF GOOD SCIENTIFIC PRACTICE* by which the research and scientific production conducted in this organisation is managed, its main objectives being to favour the improvement of science quality, and the prevention of research integrity issues. The undersigned has read and understood this code.

14. LAST FIVE YEARS REPORT ON THE SCIENTIFIC AND TECHNICAL BACKGROUND OF THE RESEARCH TEAMS:

This research proposal is driven by a multidisciplinary team of experienced researchers and ICU clinicians, whose complementary expertise has led to significant advancements in the field. The team includes specialists in Intensive Care Unit (ICU) patient care and management, respiratory infections, bacteriophages, microbiome and biofilms, microbiological diagnostics, antimicrobial resistance, and animal care and welfare during experimentation.
Dr. Laia Fernandez-Barat, the Principal Investigator (PI), has worked closely with the team for the last 5 years in this research domain. Notably, the team member Dr. Viviane de Cassia Oliveira, a recipient of the prestigious ARISTOS Postdoctoral Mobility Fellowship (MSCA), will be fully dedicated to the project. Her specialized expertise will ensure a focused and high-impact contribution, driving the achievement of the proposed objectives.
A key asset of the team is its well-established pig model of ventilator-associated pneumonia (VAP), originally developed by Prof. Antoni Torres and consolidated over the past two decades. This model has been instrumental in advancing research on novel antibiotics and therapeutic strategies for pulmonary infections and endotracheal tube biofilms. Dr. Fernandez-Barat has been involved in research using this animal model for the past 18 years, fostering strong industry partnerships to explore new preventive and therapeutic approaches for VAP and biofilm-associated respiratory infections. In particular, over the last 5 years, the PI and Prof. Torres have been working on biotherapeutic applications for VAP.

As depicted in the project organigram, the roles and responsibilities of this team based on their technical background can be organized as follows:

Transversal role:
• Dr. Laia Fernandez-Barat (PI, PhD in Clinical Microbiology in the topic antibiotherapy for Biofilms in VAP) – She will oversee work package (WP) management and coordinate the project, including meetings, reporting, scientific communication, data analysis, and publications. She will design and supervise experiments in collaboration with Dr. Oliveira and Blanca Llonch. As a Laboratory Coordinator, she ensures experimental organization, data quality, and project execution. She will also liaise with stakeholders at international meetings and industry partners, such as TechnoPhage SA.
• Prof. Antoni Torres (Emeritus Team Member) – Serving as a consultant, Prof. Torres brings extensive expertise in VAP trials and the safety of bacteriophage use. His strategic role is to align project aims with ongoing clinical trials and ensure that findings contribute to clinical guidelines and are implemented into clinical practice.

At the Microbiology Laboratory:
• Dr. Viviane de Cassia Oliveira (PhD in Microbiology, Postdoctoral Researcher) – As a leading expert in phage research, she will be primarily responsible for phage isolation, design, and characterization. She will conduct wet-lab experiments and contribute to the analysis of animal model samples to assess phage therapy efficacy, either alone or as a co-adjuvant treatment. Her extensive publication record highlights her expertise in phage manipulation.
• Dr. Roberto Cabrera (PhD in Microbiology) – He will be responsible for microbiological cultures, bacterial identification (MALDI-TOF), antimicrobial susceptibility testing, and phylogenetic analyses.
• Alba Soler (BSc in Molecular Biology, MSc in Translational Medicine) – She will manage DNA extractions, library preparations, and sequencing using MinION technology. She is also certified in animal handling and will assist in animal care during experimental VAP studies.
• Dr. Guillem Macip (BSc in Biochemistry, PhD in Bioinformatics) – As a postdoctoral researcher, he will lead biostatistics and bioinformatics analyses, ensuring robust data interpretation and computational modeling.
• Marcial Cariqueo (BSc in Pharmacy, MSc in Pharmacokinetics and Pharmacodynamics (PK/PD)) – He will contribute to safety measurements and PK/PD data analyses, supporting the assessment of treatment efficacy.
• Hired Laboratory Technician – This team member will play a pivotal transversal role in wet-lab experiments, in particular responsible for sample, strains and phage collection registry and storage.

At Animal Experimentation:
• Dr. Montserrat Rigol (Veterinarian, PhD in Medicine) – As co-responsible for the animal lab she is Certified in animal handling. She will oversee ethical compliance alongside the PI, ensuring rigorous data accuracy for analysis, scientific communications, and publications.
• Blanca Llonch (Veterinarian, PhD Student) – She is Certified in animal handling and will coordinate animal experiments, including surgical preparations, animal care, and sampling. She will also manage logistical

aspects, such as animal supplies and medications.
• Dr. Enric Barbeta (MD, PhD, Pulmonologist, ICU Faculty, Hospital Clínic) – With extensive experience in managing critically ill patients, he will contribute to animal experimentation and the collection of clinical data during experiments.
• Kasra Kiarostami (BSc in Veterinary Science, MSc in Translational Medicine) – Certified in animal handling, he will oversee animal management, medication administration, monitoring, and sampling, including sample collection at autopsy in experimental VAP models.

Some foundational studies underscore our team's ability to generate novel insights and validate innovative therapeutic strategies in preclinical models. With our strong expertise in phage and microbiome research, along with extensive experience in sophisticated animal models and biofilms, we are well-positioned to advance this research with the support of the requested grant funding.

Recent work published in the last 5 years by this team related to the topic of this proposal:

Novel Therapies: Bacteriophages for Respiratory Infections and VAP
• Oliveira VC. et al., 2024, Emerg. Microbes Infect., https://doi.org/10.1080/22221751.2024.2420737
• Mazzolini R. et al., 2023, Nat. Biotechnol., https://doi.org/10.1038/s41587-022-01584-9
• Moda-Silva LS. et al., 2022, Future Microbiol., https://doi.org/10.2217/fmb-2022-0149
Microbiome in VAP and Critically Ill Patients
• Fernández-Barat L. et al., 2020, EBioMedicine, https://doi.org/10.1016/j.ebiom.2020.102995
• Barbeta E. et al., 2024, ERJ Open Res., https://doi.org/10.1183/23120541.00667-2024
• Fernández-Barat L. et al., 2024, Chest, https://doi.org/10.1016/j.chest.2024.02.031
• López-Aladid R. et al., 2023, Sci. Rep., https://doi.org/10.1038/s41598-023-30764-z
• Barbeta E. et al., 2023, Crit Care., https://doi.org/10.1186/s13054-023-04512-8
Antimicrobial Resistance, Administration Modes in VAP, and Other Respiratory Infections
• Motos A. et al., 2021, Antimicrob. Agents Chemother., https://doi.org/10.1128/AAC.01899-20
• Cabrera R. et al., 2025, Scientific Reports, https://doi.org/10.1038/s41598-025-91368-3
• Cabrera R. et al., 2022, J Antimicrob Chemother., https://doi.org/10.1093/jac/dkac084
• Cabrera R. et al., 2020, Antimicrob Resist Infect Control., https://doi.org/10.1186/s13756-020-0679-z
• Motos A. et al., 2023, Crit Care., https://doi.org/10.1186/s13054-023-04331-x

The research team combines expertise from clinical, microbiological, veterinary, PK/PD, computational and data analyses fields, creating a highly interdisciplinary and gender-balanced research environment. This synergy drives scientific excellence and fosters innovation in phage therapy, antimicrobial resistance, and respiratory infection treatment. With a track record of high-impact publications, extensive experience in preclinical and translational research, and strong industry collaborations, the team is uniquely positioned to develop transformative therapeutic strategies that can bridge the gap between bench and bedside in VAP treatment.

15. STATE UP TO FIVE PUBLICATIONS (IN THE FIELD OF Respiratory Diseases) IN HIGH Q1 JOURNALS OR TWO PUBLICATIONS IN FIRST OR LAST POSITION (IN THE FIELD OF Respiratory Diseases) IN HIGH Q1 JOURNALS, WRITTEN BY THE PI IN THE PAST 5 YEARS:

Article title. Authors, complete title, journal, year, volume and pages:

Oliveira VC, Soler-Comas A, Rocha ACSD, Silva-Lovato CH, Watanabe E, Torres A, Fernández-Barat L (Corresponding author). The synergistic effect between phages and Ceftolozane/Tazobactam in Pseudomonas aeruginosa endotracheal tube biofilm. Emerg Microbes Infect. 2024 Dec;13(1):2420737. http://doi.org/10.1080/22221751.2024.2420737

Impact Factor/ Citation (last recount):

8.4/0

Abstract:

Although an increased effectiveness has been suggested when phages and antibiotics are combined, this approach has not been tested against a mature biofilm on an endotracheal tube (ETT) surface. This study evaluated the effect of short- and long-term combined phage-antibiotic therapy in a control of a mature biofilm on an ETT surface. Pseudomonas aeruginosa strains, including susceptible and resistant clinical samples, were used to develop the ETT biofilm. Biofilm was treated with 108PFU/mL of phage_2, phage_18 or 5 µg/mL of ceftolozane/tazobactam, alone or in combination with phages. The sequential combination of the two different phages and ceftolozane/tazobactam was also tested. Biofilm viability was assessed after short (2, 4, 24 h) and long-(48, 72 h) term treatment exposure using colony forming unit measurement. For long-term exposition, a new treatment shot was added every 24 h. In the sequential combination, the phage type was switched at 24 h of treatment. Regarding the susceptible strains, the treatments had limited antibiofilm effect after 2, 4 and 24 h. After 48 and 72 h, administering phages alone had no effect on biofilm viability, indicating the emergence of phage-resistant phenotypes. Nonetheless, the combined phage-antibiotic treatment reduced the biofilm viability in about 5-log, whilst antibiotic alone reduced in about 3-log. The sequential combination of phages and antibiotic reduced the biofilm viability in about 6-log. With respect to the resistant strains, no antibiofilm activity was observed regarding the treatment arms. The combination of phages and ceftolozane/tazobactam showed a synergism strain-dependent, being more apparent in susceptible strains.

Article title. Authors, complete title, journal, year, volume and pages:

Fernández-Barat L (Corresponding author), López-Aladid R, Torres A. Reconsidering ventilator-associated pneumonia from a new dimension of the lung microbiome. EBioMedicine. 2020 Oct;60:102995. http://doi.org/10.1016/j.ebiom.2020.102995.

Impact Factor/ Citation (last recount):

9.7/25

Description:

Complex microbial communities that reside in the lungs, skin and gut are now appreciated for their role in maintaining organ, tissue and immune homoeostasis. As lungs are currently seen as an ecosystem, the shift in paradigm calls for the consideration of new algorithms related to lung ecology in pulmonology. Evidence of lung microbiota does not solely challenge the traditional physiopathology of ventilator-associated pneumonia (VAP); indeed, it also reinforces the need to include molecular techniques in VAP diagnosis and accelerate the use of immunomodulatory drugs, including corticosteroids, and other supplements such as probiotics for VAP prevention and/or treatment. With that stated, both microbiome and virome, including phageome, can lead to new opportunities in further understanding the relationship between health and dysbiosis in VAP. Previous knowledge may be, however, reconsidered at a microbiome scale.

Article title. Authors, complete title, journal, year, volume and pages:

Galli F, Bindo F, Motos A, Fernández-Barat L. (Corresponding author), et al. Procalcitonin and C-reactive protein to rule out early bacterial coinfection in COVID-19 critically ill patients. Intensive Care Med. 2023 Aug;49(8):934-945. http://doi.org/10.1007/s00134-023-07161-1

Impact Factor/ Citation (last recount):

29.6/16

Description:

Purpose: Although the prevalence of community-acquired respiratory bacterial coinfection upon hospital admission in patients with coronavirus disease 2019 (COVID-19) has been reported to be < 5%, almost three-quarters of patients received antibiotics. We aim to investigate whether procalcitonin (PCT) or C-reactive protein (CRP) upon admission could be helpful biomarkers to identify bacterial coinfection among patients with COVID-19 pneumonia.

Methods: We carried out a multicentre, observational cohort study including consecutive COVID-19 patients admitted to 55 Spanish intensive care units (ICUs). The primary outcome was to explore whether PCT or CRP serum levels upon hospital admission could predict bacterial coinfection among patients with COVID-19 pneumonia. The secondary outcome was the evaluation of their association with mortality. We also conducted subgroups analyses in higher risk profile populations.

Results: Between 5 February 2020 and 21 December 2021, 4076 patients were included, 133 (3%) of whom presented bacterial coinfection. PCT and CRP had low area under curve (AUC) scores at the receiver operating characteristic (ROC) curve analysis [0.57 (95% confidence interval (CI) 0.51-0.61) and 0.6 (95% CI, 0.55-0.64), respectively], but high negative predictive values (NPV) [97.5% (95% CI 96.5-98.5) and 98.2% (95% CI 97.5-98.9) for PCT and CRP, respectively]. CRP alone was associated with bacterial coinfection (OR 2, 95% CI 1.25-3.19; p = 0.004). The overall 15, 30 and 90 days mortality had a higher trend in the bacterial coinfection group, but without significant difference. PCT ≥ 0.12 ng/mL was associated with higher 90 days mortality.

Conclusion: Our study suggests that measurements of PCT and CRP, alone and at a single time point, are not useful for ruling in or out bacterial coinfection in viral pneumonia by COVID-19.

Article title. Authors, complete title, journal, year, volume and pages:

Kiarostami K*, Fernández-Barat L* (Corresponding and co-first author), Battaglini D, Motos A, Bueno-Freire L, Soler-Comas A, Bassi GL, Torres A. The efficacy of telavancin in comparison with linezolid on endotracheal tube biofilm in pigs with methicillin-resistant Staphylococcus aureus pneumonia. Int J Antimicrob Agents. 2024 Feb;63(2):107052. http://doi.org/10.1016/j.ijantimicag.2023.107052.

Impact Factor/ Citation (last recount):

4.9/1

Description:

Background: The effect of systemic treatment of ventilator-associated pneumonia (VAP) with telavancin, a semisynthetic lipoglycopeptide with good penetration in vitro biofilms, has not been tested in vivo during mechanical ventilation. This study examined the efficacy of telavancin compared with linezolid against endotracheal tube (ETT) biofilms in a porcine model of methicillin-resistant Staphylococcus aureus (MRSA) VAP.

Methods: VAP was induced in 18 pigs by instilling 107 colony-forming units (CFU/mL) of an MRSA strain susceptible to telavancin and linezolid into each pulmonary lobe. Randomization into three groups was done at pneumonia diagnosis: control (IV glucose 0.5% solution q24); linezolid (10 mg/kg q12) and telavancin groups (22.5 mg/kg q24). After 72 h of MV, data regarding bronchoalveolar lavage (BAL), tracheal aspirate (TA), ETT MRSA biofilm load and thickness measured by scanning electron microscopy were obtained.

Results: All 18 pigs completed the study. MRSA was isolated in 100% of ETTs from the control and linezolid groups and in 67% from the telavancin group. Telavancin treatment presented a lower MRSA load compared to the control and linezolid treatments (telavancin median [interquartile range (IQR)] = 1.94 [0.00-5.45], linezolid 3.99 [3.22-4.68] and control 4.93 [4.41-5.15], P = 0.236). Telavancin treatment also resulted in the lowest biofilm thickness according to the SEM (4.04 [2.09-6.00], P < 0.001). We found a positive correlation between ETT and BAL load (rho = 0.511, P = 0.045).

Conclusions: In our VAP model, systemic telavancin treatment reduced ETT MRSA occurrence, load, and biofilm thickness. Our findings may have a bearing on ICU patients' clinical outcomes.

Article title. Authors, complete title, journal, year, volume and pages:

Cabrera R*, Fernández-Barat L* (Corresponding and co-first author), Motos A, López-Aladid R, Vázquez N, Panigada M, Álvarez-Lerma F, López Y, Muñoz L, Castro P, Vila J, Torres A. Molecular characterization of methicillin-resistant Staphylococcus aureus clinical strains from the endotracheal tubes of patients with nosocomial pneumonia. Antimicrob Resist Infect Control. 2020 Feb 28;9(1):43. http://doi.org/10.1186/s13756-020-0679-z.

Impact Factor/ Citation (last recount):

4.8/19

Description:

Background: The objective of our study was to determine the antimicrobial susceptibility, the associated molecular mechanisms of resistance and the epidemiological relatedness of MRSA strains isolated from the endotracheal tubes (ETT) of intubated critically ill patients in the intensive care unit (ICU) with nosocomial pneumonia caused by Staphylococcus aureus.
Methods: The antimicrobial susceptibility to vancomycin, linezolid, ciprofloxacin, clindamycin, erythromycin, chloramphenicol, fusidic acid, gentamicin, quinupristin-dalfopristin, rifampicin, sulfamethoxazole/trimethoprim, and tetracycline were measured. Resistance mechanisms were then analyzed by polymerase chain reaction and sequencing. Molecular epidemiology was carried out by multi-locus sequence typing.
Results: S. aureus isolates were resistant to ciprofloxacin, erythromycin, gentamicin, tetracycline, clindamycin, and fusidic acid. The most frequent mutations in quinolone-resistant S. aureus strains were S84L in the gyrA gene, V511A in the gyrB gene, S144P in the grlA gene, and K401R/E in the grlB gene. Strains resistant to erythromycin carried the ermC, ermA, and msrA genes; the same ermC and ermA genes were detected in strains resistant to clindamycin. The aac(6')-aph(2″) gene was related to gentamicin resistance, while resistance to tetracycline was related to tetK (efflux pump). The fusB gene was detected in the strain resistant to fusidic acid. The most frequent sequence types were ST22, ST8, and ST217, which were distributed in four clonal complexes (CC5, CC22, CC45, and CC59).
Conclusions: High levels of resistance to second-line antimicrobials threatens the treatment of nosocomial respiratory infections due to methicillin-resistant S. aureus with decreased susceptibility to linezolid and vancomycin. The wide genotypic diversity found reinforces the central role of ICU infection control in preventing nosocomial transmission.

RESEARCH TEAM:

Organisation:	Fundació de Recerca Clínic Barcelona - Institut d'Investigacions Biomèdiques August Pi i Sunyer	**Family name:**	Fernandez Barat
Centre:	Fundació de Recerca Clinic Barcelona - Institut d'Investigacions Biomèdiques August Pi i Sunyer	**First name:**	Laia
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		Degrees:	Doctor

Signature:

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Centre:	Centro de Investigación Biomédica en Red (CIBER), Enfermedades Respiratorias (CIBERES)	**First name:**	Viviane
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		Degrees:	Doctor

Signature:

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		Degrees:	Doctor

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		Degrees:	Llicenciat

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Organisation:	Fundació de Recerca Clínic Barcelona - Institut d'Investigacions Biomèdiques August Pi i Sunyer	**Family name:**	Cabrera
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Organisation:	Fundació de Recerca Clínic Barcelona - Institut d'Investigacions Biomèdiques August Pi i Sunyer	**Family name:**	Torres Martí
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Centre:	Fundació de Recerca Clínic Barcelona - Institut d'Investigacions Biomèdiques August Pi i Sunyer	**First name:**	Blanca
		NIF:	47947598G
		Degrees:	Llicenciat

Signature:

17. OTHER RESEARCH GROUPS

Dr. Maria Tomás (Spain) leads WP3 (Bacteriophage Therapy) in the MEPRAM project and holds a JPIAMR grant, both focused on antimicrobial resistance. Dr. Jean-Paul Pirnay (Belgium) pioneered clinical phage therapy, treating 100 patients, and advises this project. Drs. Joana Azeredo & Sanna Sillankorva (Portugal) contributed to phage therapy authorization and ESCMID's biofilm group. Dr. Watanabe (Brazil) studies phage therapy for oral dysbiosis, collaborating with the PI's team. The ESGHAMI group (ESCMID) explores microbiota-based therapies, including phage therapy (Lancet Microbe, 2025). The yearly congress "Targeting Phage Therapy", gathers researchers, industries and policy makers on phage therapy.

DATA FROM THE PRINCIPAL INVESTIGATOR

FAMILY NAME: Fernandez Barat
FIRST NAME: Laia
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BIRTH DATE: 20/01/1979
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DEPARTMENT: Applied Research in Respiratory Infectious Diseases and Critically ill patients
EMAIL: Lfernandez79@ub.edu

ACADEMIC TRAINING:

Batchelor degree / Engineering:	Biology, speciality: Biomedical Sciencies	**Date:**	05/09/2002
Doctorate:	Clinical Microbiology	**Date:**	18/07/2013
Dissertation Director:	Prof. Antoni Torres Martí and Dr. Miquel Ferrer Monreal		

PROFESSIONAL STATE:

Professional status:	Laboratory Coordinator-Associate Professor of Microbiology	**Start date:**	01/01/2013
Dedication:	FullTime		
Working situation:	Hired		

1. PREVIOUS SCIENTIFIC OR PROFESSIONAL ACTIVITIES

2013 – Present: Laboratory Coordinator (Certificate R3), Associate Professor, Department of Microbiology, Faculty of Pharmacy, University of Barcelona.

2014 – 2016: Postdoctoral project, including an international stay at Rigshospitalet-Costerton Biofilm Centre, Copenhagen (Denmark). Dr. Laia Fernández-Barat was awarded a joint fellowship (STRTF 2014-4911) from the European Respiratory Society (ERS) and the Spanish Society of Pneumology and Thoracic Surgery (SEPAR).

2007 – 2013: Predoctoral Fellow and Project Manager.

2003 – 2007: Scientific Communication at multiple institutions:

Hospital Clínic – Scientific Communication Office (2003–2004).
CosmoCaixa – Scientific Education Department (2005–2006).
Parc de Recerca Biomèdica de Barcelona (PRBB) – Scientific Communication Office (2006–2007).
Freelance Scientific Journalist for Societat Catalana de Biologia, organizing outreach activities for students ("Viu La Ciència Contemporània").
El Temps – Journalist specializing in scientific content.

2. PARTICIPATION IN RESEARCH PROJECTS FINANCED IN THE LAST FIVE YEARS

1. Project title: Long-term alterations of host-microbiome interactions and cardiovascular and respiratory diseases progression after pneumonia. Agency: H2020, European Comission.The homi-lung project Funding: 6.998.420 euros. Period: 2024-28. Principal Investigator: Antoine Roquilly (University of Nantes, France), Laia Fernández Barat as Team Member and WP7 co-leader with Prof.Torres
2.Project title: Combined efficacy of antibiotics and bacteriophages for the personalized treatment of ventilator-associated pneumonia. Agency: SEPAR. Funding: 12.000 euros. Period: 2025-27. Principal researcher: Laia Fernández Barat
3. Project title: Influence of Pseudomonas aeruginosa Pathogenesis on the Immune Response and Microbiome during Exacerbation and Stable Phases. Agency: SEPAR. Funding: 9.000 euros. Period: 2023-25. Principal researcher: Laia Fernández Barat
4.Project title: The diagnostic value of the molecular platforms and microbiome in ventilator-associated lower respiratory tract infections: Its impact on clinical outcomes in COVID and non-COVID patients. Agency: Instituto de Salud Carlos III (ISCIII)/Biomerieux. Funding: 123.000 euros. Period: 2021-23. Principal researcher: Laia Fernández Barat
5. Project Title: Personalized medicine in respiratory infections and the critically ill patient with respiratory failure. Agency: Generalitat de Catalunya. Funding: 60.000 euros. Period: 2022-24. Principal researcher: Laia Fernández Barat
6.Project title: "Human recombinant interferon gamma-1b for the prevention of hospital-acquired pneumonia in critically ill patients: a double-blind, international, phase 2, randomized, placebo-controlled trial - the PREV-HAP study" (Eudract: 2020-000620-18/Ref: RC20_0082) Agency: European Comission (H2020). Funding: 9.996.350 euros. Period: 2020-24. Principal Researcher: Antoine Roquilly (University of Nantes), Antoni Torres as WP2 leader; Laia Fernandez's Role: Team Member
7. Project title: Identification of epitopes and isolation of IgG anti-COVID-19 to produce monoclonal antibodies for treatment. Agency: Departament General de Recerca e Innovació en Salut (DGRIS-Gencat). Funding: 379.000 euros. Period: 2020-21. European Patent: EP22382470.7 (16/05/2022, Title: SARS-CoV-2 immunogenic polypeptide, anti- SARS-CoV-2-spike glycoprotein antibodies and antigen-binding fragments). Principal researcher: Laia Fernández Barat
8. Project title: «Factores de riesgo y pronóstico personalizados y seguimiento a un año de los enfermos ingresados en las unidades de cuidados intensivos españolas infectados por el virus SARS-CoV-2» (CIBERESUCICOVID). Agency: Instituto de Salud Carlos III (ISCIII). Funding: 1.750.000 euros. Period: 2020-21. Principal researcher: Antoni Torres Martí; Laia Fernandez's Role: Member of the Steering Committee
9. Project title: Bacterial lung chassis for treating human lung infectious diseases. Agency: “La Caixa” Health Research Call 2018. Funding: 999.828 euros. Principal researchers: Luis Serrano (Center for Genomic Regulation)/Antoni Torres Martí; Laia Fernandez's Role: Coordinator of Biofilm studies
10. Project title: Role of Pseudomonas aeruginosa biofilms in exacerbations in patients with bronchiectasis with and without Chronic Obstructive Pulmonary Disease. Agency: Proyectos en Salud (FIS)- Instituto de Salud Carlos III, Centro de Investigación Biomédica en Red (CIBER), Spanish Society of Pneumology and Thoracic Surgery (SEPAR). Funding: 274.000 euros. Period: 2019-21. Principal researcher: Laia Fernández Barat/Antoni Torres Martí

3. PUBLICATIONS (in the last five years, and up to 10 publications)

1- O (Q1)
Cabrera R, Rovira-Ribalta N, Motos A, Bueno-Freire L, Vázquez N, Soler-Comas A, Alcaraz-Serrano V, López-Aladid R, Muñoz L, Vila J, Torres A, Fernández-Barat L (Corresponding author, CA). Virulence factors of Pseudomonas aeruginosa and immune response during exacerbations and stable phase in bronchiectasis. Sci Rep. 2025 Feb 22;15(1):6520. http://doi.org/10.1038/s41598-025-91368-3

2- O (Q1)
Oliveira VC, Soler-Comas A, Rocha ACSD, Silva-Lovato CH, Watanabe E, Torres A, Fernández-Barat L (CA). The synergistic effect between phages and Ceftolozane/Tazobactam in Pseudomonas aeruginosa endotracheal tube biofilm. Emerg Microbes Infect. 2024 Dec;13(1):2420737. http://doi.org/10.1080/22221751.2024.2420737

3- O (Q1)
Kiarostami K*, Fernández-Barat L* (Co-first and CA), Battaglini D, Motos A, Bueno-Freire L, Soler-Comas

A, Bassi GL, Torres A. The efficacy of telavancin in comparison with linezolid on endotracheal tube biofilm in pigs with methicillin-resistant Staphylococcus aureus pneumonia. Int J Antimicrob Agents. 2024 Feb;63(2):107052.
https://doi.org/10.1016/j.ijantimicag.2023.107052

4- O (Q1)
Fernández-Barat L (CA), Vázquez Burgos N, Alcaraz V, Bueno-Freire L, López-Aladid R, Cabrera R, Gabarrús A, Palomeque A, Oscanoa P, Ceccato A, Motos A, Amaro R, Bernardi T, Provot C, Soler-Comas A, Muñoz L, Vila J, Torres A. The value of biofilm testing to guide antimicrobial stewardship in chronic respiratory diseases. Front Cell Infect Microbiol. 2023 May 2;13:1142274.
https://doi.org/10.3389/fimmu.2023.1278534

5- O (Q1)
Galli F, Bindo F, Motos A, Fernández-Barat L (CA), Barbeta E, Gabarrús A, Ceccato A, Bermejo-Martin JF, Ferrer R, Riera J, Peñuelas O, Lorente JÁ, de Gonzalo- Calvo D, Menéndez R, Gonzalez J, Misuraca S, Palomeque A, Amaya-Villar R, Añón JM, Balan Mariño A, Barberà C, Barberán J, Blandino Ortiz A, Bustamante-Munguira E, Caballero J, Cantón-Bulnes ML, Carbajales Pérez C, Carbonell N, Catalán-González M, de Frutos R, Franco N, Galbán C, Lopez Lago A, Gumucio-Sanguino VD, de la Torre MDC, Díaz E, Estella Á, Gallego Curto E, García-Garmendia JL, Gómez JM, Huerta A, Jorge García RN, Loza-Vázquez A, Marin-Corral J, Martin Delgado MC, Martínez de la Gándara A, Martínez Varela I, Lopez Messa J, M Albaiceta G, Nieto MT, Novo MA, Peñasco Y, Pérez-García F, Pozo-Laderas JC, Ricart P, Sagredo V, Sánchez-Miralles A, Sancho Chinesta S, Roche-Campo F, Socias L, Solé-Violan J, Suarez-Sipmann F, Tamayo Lomas L, Trenado J, Úbeda A, Valdivia LJ, Vidal P, Boado MV, Rodríguez A, Antonelli M, Blasi F, Barbé F, Torres A; CIBERESUCICOVID Project investigators (COV20/00110, ISCIII). Procalcitonin and C-reactive protein to rule out early bacterial coinfection in COVID-19 critically ill patients. Intensive Care Med. 2023 Aug;49(8):934-945.
https://doi.org/10.1007/s00134-023-07161-1

6- O (Q1)
Farriol-Duran R, López-Aladid R, Porta-Pardo E, Torres A, Fernández-Barat L. Brewpitopes: a pipeline to refine B-cell epitope predictions during public health emergencies. Front Immunol. 2023 Dec 6;14:1278534.
https://doi.org/10.3389/fimmu.2023.1278534

7- O (Q1)
Enric Barbeta, Rubén López-Aladid, Letícia Bueno-Freire, Blanca Llonch, Andrea Palomeque, Anna Motos, Ricard Mellado-Artigas, Luigi Zattera, Carlos Ferrando, Alba Soler, Laia Fernández-Barat* (CA), Antoni Torres*. Biological effects of pulmonary, blood and gut microbiome alterations in patients with acute respiratory distress syndrome. ERJ Open Research 2024 00667-2024.
https://doi.org/10.1183/23120541.00667-2024

8- O (Q1)
Cabrera R*, Fernández-Barat L* (Co-first and CA), Vázquez N, Alcaraz-Serrano V, Bueno-Freire L, Amaro R, López-Aladid R, Oscanoa P, Muñoz L, Vila J, Torres A. Resistance mechanisms and molecular epidemiology of Pseudomonas aeruginosa strains from patients with bronchiectasis. J Antimicrob Chemother. 2022 May
29;77(6):1600-1610.
https://doi.org/10.1093/jac/dkac084

9- A (Q1)
Fernández-Barat L (CA), López-Aladid R, Torres A. Reconsidering ventilator-associated pneumonia from a new dimension of the lung microbiome. EBioMedicine. 2020 Oct;60:102995.
https://doi.org/10.1016/j.ebiom.2020.102995

10- O (Q1)
Cabrera R*, Fernández-Barat L* (Co-first and CA), Motos A, López-Aladid R, Vázquez N, Panigada M, Álvarez-Lerma F, López Y, Muñoz L, Castro P, Vila J, Torres A. Molecular characterization of methicillin-resistant Staphylococcus aureus clinical strains from the endotracheal tubes of patients with nosocomial pneumonia. Antimicrob Resist Infect Control. 2020 Feb 28;9(1):43. https://doi.org/10.1186/s13756-020-0679-z

4. PATENTS AND UTILITY MODELS (in the last ten years)

Title: SARS-COV-2 IMMUNOGENIC POLYPEPTIDE, ANTISARS-COV-2-SPIKE GLYCOPROTEIN ANTIBODIES AND ANTIGEN-BINDING FRAGMENTS
Inventors: Laia Fernandez Barat (CIBER); Antoni Torres Martí (HCB-IDIBAPS); Ruben López Aladid (IDIBAPS); Carlota Dobaño Lázaro (ISGlobal); Gemma Moncunill Piñas (ISGlobal); Eduard Porta (BSC); Roc Farriol Duran (BSC)
Request number: EP22382470.7.
Priority Country: Spain.
Priority Date: 16/05/2022.
Applicants (Entities): UNIVERSITAT DE BARCELONA; CONSORCIO CENTRO DE INVESTIGACIÓN BIOMÉDICA EN RED; HOSPITAL CLÍNIC DE BARCELONA; INSTITUT D'INVESTIGACIONS BIOMÈDIQUES AUGUST PI I SUNYER, FUNDACIÓN PRIVADA INSTITUTO DE SALUD GLOBAL BARCELONA; BARCELONA SUPERCOMPUTING CENTER -CENTRO NACIONAL DE SUPERCOMPUTACIÓN
Non extended to countries
No companies exploiting it

5. STAYS IN FOREIGN CENTRES (continued stays longer than six months in the last five years)

Postdoctoral stay at Rigshospitalet – Costerton Biofilm Centre, Copenhagen (Denmark) in 2014 (prior to the last five years), awarded through an ERS-SEPAR fellowship, no more than 6 months stay.