**PROJECT CO-FUNDED BY EUROPEAN REGIONAL DEVELOPMENT FUNDS (ERDF)**

**Lead Researcher:** Dr. Carmen del Águila de la Puente

**Reference**: PI17/01670

**Title**: Pneumonia in the communitary and nosocomial field: Acamthamoeba as a refuge and training camp of Legionella. Development of new diagnostic methods for its implementation (Neumonía en el ámbito comunitario y nosocomial: *Acanthamoeba* como refugio y campo de entrenamiento de *Legionella*. Desarrollo de nuevas herramientas diagnósticas para su traslación).

**Financing Entity:** Spanish Ministry of Economy, Industry and Competitivity (Ministerio de Industria, Economía y Competitividad); Carlos III Health Institute (Instituto de Salud Carlos III).

**Total amount**: 87.120 €

**Start date**: 01/01/2018

**End date**: 01/01/2018

**Summary:**

**MAIN PURPOSE:**

Characterisation of *Acanthamoeba*’s vectorial role as reservoir of *Legionella spp* and its effect on: a) the acquisition of infectious pathologies in the field of the community and nosocomial environment, b) the modulation of virulence and interaction factors with the host, contributing to an improvement of the diagnosis through the development of new tools for its implementation into the health field.

**SPECIFIC PURPOSES:**

1. To isolate and characterise Free Living Amoebae (FLA) and *Legionella* in clinical and hospital water samples, studying the molecular epidemiology of these pathogens as agents responsible for pneumonia.
2. To study the pathogen/host interaction in the selected microorganisms: factors associated with invasion/colonisation, virulence factors, Immune System Evasion Mechanisms. “In vitro” model.
3. To develop new alternatives for the diagnosis of pneumonia caused by *Legionella spp,* useful in hospitals for the simultaneous detection of the different species of *Legionella*, including *L. no-pneumophila*.

**METHODOLOGY:**

Organisms shall be recovered by filtration from the different types of water and pathogens shall be identified by culture and PCR. Clinical samples will be analysed by culture, molecular methods and immunochromatography. Regarding the rest of the activities and tasks, the following methodologies will be developed: aptamer-based technology, electrophoresis, molecular characterisation. Animal models and anatomopathological studies.

**PROJECT DESIGN AND TASKS:**

**Project design**

Study phases:

1. Extraction of samples from different origins.
2. Identification, enlargement and characterisation of the microorganisms in the different samples.
3. Study of pathogen/host interaction.
4. Improvement of the pneumonia diagnosis.
5. Analysis data and results treatment.

**Task to carry out**

* **TASK 1- IDENTIFICATION, CHARACTERISATION AND CULTURE OF MICROORGANISMS COLLECTED IN THE WATER AND CLINICAL SAMPLES.**

# Activity 1.1.- Extraction of biological reference material from the different protozoa and bacteria strains within the study.

# Activity 1.2.- Design the sample collection plan.

# Activity 1.3.- Identification and characterisation of mechanisms collected in water samples.

# Activity 1.4 Identification and characterisation of FLA and Legionella associated with clinical samples.

* **TASK 2: STUDY OF THE PATHOGEN-HOST INTERACTION AND CHARACTERISATION OF THE FACTORS ASSOCIATED TO THE INVASION/COLONISATION OF *Acanthamoeba* by *Legionella*.**

# Activity 2.1: Immune System Evasion Mechanisms and *Legionella* Virulence Factors.

* **TASK 3: OBTENTION OF USEFUL TOOLS FOR THE DEVELOPMENT OF A SPECIFIC AND LOW-COST DIAGNOSTIC METHOD OF THE LEGIONELLOSIS.**

# Activity 3.1: Development of specific aptamers for the different species of *Legionella spp*.

# Activity 3.2: Study of aptamers’ specificity and functionality.

* **TASK 4: EXCHANGE OF RESEARCHERS AND TECHNIQUES**
* **TAST 5: ANALYSIS AND PUBLICATION OF THE RESULTS OBTAINED**

**PROJECT ORGANISATION:**

The **first stage** will focus on the extraction of biological reference material, the optimisation of molecular identification methods and the design of the sample collection plan.

In the **second stage**, in its first phase, sampling will be carried out at the selected places and times, identifying the pathogens under study in both water samples and clinical samples and starting the characterisation. At the same time, the corresponding FLA strains will be cultured and characterisation assays for the vectorial role of *Acanthamoeba* will be started. In addition, the design of alternative diagnostic methods will be started. The second phase of this second stage will mainly involve molecular characterisation of isolates from water and clinical samples, proteomic characterisation of receptors and pathogen-host interaction and animal model studies.

A key point in this project is to have the collaboration of NHS hospitals such as Hospital Puerta de Hierro and Hospital La Paz, which will provide us with both paediatric and adult clinical samples, and Hospital Gregorio Marañón, whose technology (Flow Cytometry) and Biobanks Platform will be available to us. On the other hand, all the water sampling from different hospital units will be carried out thanks to the collaboration with the Hospital Arnau de Vilanova in Valencia and the Hospitales Virgen del Rocío, Hospital Esperanza Macarena and Hospital Nuestra Señora de Valme.

**For reasons related to the call**, members of the collaborating centres are not included in the distribution of tasks, but their participation is listed as "place of execution". In order to facilitate their identification, they have been marked with \*. Although they are not part of the research group, their collaboration is essential for the project: Dr. Coral Barbas Arriba, Director of Universidad San Pablo-CEU Centre of Excellence in Metabolomics and Bioanalysis (collaboration in the development of the aptamer by selection with Capillary Electrophoresis and offering us all their Mass Spectrophotometry service) and Dr. J. Carlos Andreu from the Hospital Arnau de Vilanova (sending hospital water samples), Dr. Mª José Mellado, head of the paediatrics service at the Hospital de la Paz (coordination and paediatric consultancy) and Dr. Mª Ángeles Muñoz Fernández, head of the Biobank at the Hospital Gregorio Marañón (consultancy).

**Staff**: CA: Carmen del Águila (USP-CEU); SF: Soledad Fenoy (USP-CEU); CH: Carolina Hurtado (USP-CEU); DO: Dolores Ollero (USPCEU); AM: Angela Magnet (USP-CEU); CS: Contrato solicitado (USP-CEU); LA: Luis Arvelo (HPH); IS: Isabel Sánchez (HPH); SP: Sara Pérez (HLP); MP: Manuela de Pablos (HLP); LL: Luis Lopez (HVR y HEM); CG: Coral Gómez (HGM).

**Centres**: USPCEU: Universidad San Pablo-CEU; HPH: Hospital Puerta de Hierro; HLP: Hospital La Paz; HVR: Hospital Virgen del Rocío; HEM: Hospital Esperanza Macarena; HGM: Hospital de Gregorio Marañón.

**Collaborating Centres and Staff:** CEMBIO\*: Metabolomics and Bioanalysis Excellence Centre; HAV\*: Hospital Arnau de Vilanova.

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