APPEL D'OFFRE FONDATION MEDITERRANEE INFECTION 2024

DOCTORANT H/F

Titre du projet en français et en anglais :

Evaluation de la sensibilité aux antibiotiques de *Coxiella burnetii* par microscopie électronique à balayage

Evaluation of Coxiella burnetii antibiotic susceptibility by scanning electron microscopy

Descriptif du projet en 10 lignes en Français/Anglais (abstract) :

La fièvre Q, causée par la bactéries intracellulaire *Coxiella burnetii*, est une zoonose de plus en plus préoccupante pour la santé publique **(Turcotte, M.-È *et al.*, 2021) (Cléa Melenotte *et al.*, 2019).** L'infection à *Coxiella burnetii* demeure un défi pour les médecins, principalement en raison de l'absence de coordination internationale pour améliorer les résultats cliniques et les tests de sensibilité aux antibiotiques **(Cléa Melenotte *et al.*, 2019).** L'émergence de la résistance a été associée à des épidémies, notamment dans les cas de rechute et de fièvre Q chronique **(Aupee O *et al.*, 2009).**

L'objectif de notre recherche est de développer une méthode d'étude de sensibilité aux antibiotiques en utilisant la microscopie électronique. Dans le but de standardiser ce test et de l'appliquer directement aux échantillons afin de pouvoir fournir des résultats reproductibles entre les différents laboratoires et dans un délai inférieur à celui requis par les méthodes classiques. De plus, nous prévoyons intégrer une analyse génomique car il existe actuellement un manque d'études génomiques approfondies sur la résistance de *Coxiella burnetii*.

Q fever, a disease caused by the intracellular bacterium *Coxiella burnetii*, is a zoonosis of increasing public health concern **(Turcotte, M.-È *et al.*, 2021) (Cléa Melenotte *et al.*, 2019)**. *Coxiella burnetii* infection remains a constant challenge for physicians, mainly due to the lack of international coordination to improve clinical outcomes and appropriate antibiotic susceptibility testing. The emergence of resistance has been associated with epidemics, particularly in relapsing and chronic Q fever **(Aupee O et al., 2009).**

The objective of our research is to develop a method for studying antibiotic susceptibility using electron microscopy. The aim is to standardize this test and apply it directly to samples, in order to provide results in a shorter timeframe than that required by conventional methods. In addition, we plan to integrate a genomic analysis, as there is currently a lack of in-depth genomic studies on *Coxiella burnetii* resistance

Introduction et état de l'art :

Coxiella burnetii, an intracellular bacterium, is the causative agent of Q fever, a disease that has a polymorphic presentation in human **(Raoult D *et al.*, 2005) (Melenotte C *et al.*, 2020)**. We differentiate acute Q fever and persistent focalized infection. The first, ranges from an asymptomatic seroconversion to more severe symptoms including pneumoniae and hepatitis **(Caron F *et al.*,1998)(Fournier PE *et al.*, 2001).** In persistent focalized infection, endocarditis is the most frequent clinical presentation. Regarding the treatment, doxycycline is the recommended first line therapy. However, some *C. burnetii* strains are doxycycline-resistant, and cases of acquired resistance to doxycycline during prolonged treatment, as is the case for Q fever endocarditis, have been reported **(Eldin C *et al.*,2016)**. Currently, antibiotic susceptibility testing for *C. burnetii* is a challenge, being

limited to few laboratories worldwide due to the major limitations of culture on cellular and axenic media and the requirement to work in BSL3 environment **(Mori M *et al.,* 2017)**. In addition, among the hurdles standing in the way of such testing is the fact that *C. burnetii* is a slow-growing and fastidious bacterium, making it impossible to accurately assess its antibiotic susceptibility **(Diana J Vaca *et al.,* 2022)** and to give results in less than two to three weeks**.** In addition, the variability of test protocols used in clinical and research laboratories means that there is a lack of standardization, making it difficult to compare results between studies **(Eldin C *et al.,* 2017).**

In the present project, we aim to use scanning electron microscopy for in-depth study of antibiotic susceptibility testing (AST) of *C. burnetiid* **(Gabriel Haddad *et al.,* 2021)**. This approach involves the culture of susceptible (Nine Mile strain) and resistant (Henzerling strain) *C. burnetii* strains, which will be exposed to various antibiotics to assess their efficacy against the bacterium **(K. A. Clay *et al.,* 2021)**. After treatment, both strains will be prepared for microscopic observation, enabling direct visualization of the effects of antibiotics on bacterial cell morphology and structure, thus enabling to provide results for antibiotic susceptibility testing within a few days. Quantitative analyses will also be carried out to quantify the effects of antibiotics on *C. burnetii* growth and viability, using PCR and genome sequencing **(GB Howe, *et al.,* 2009)**.

Using this microscopy approach, we aim to obtain detailed data on the response of *Coxiella* to antibiotics in a shorter time compared to traditional methods, enabling a better understanding of resistance and susceptibility mechanisms, and the optimization of treatment strategies by obtaining rapid AST results and by testing a panel of antibiotics

Matériels et méthodes : (confidentiel data)

-Strain collection

Coxiella burnetii strains will be provided by the national reference center (CNR). The panel of strains will include susceptible (Nine Mile...) and resistant strains (Henzerling…) to doxycycline.

-Culture

Strains will be cultivated on axenic media (cell free media) within the NSB3 laboratory.

-Microscopic Observation

Electron microscopy observation will be done on a table top scanning electron microscope with the collaboration of the microscopy team. This will allow direct visualization of the effects of antibiotics on bacterial growth cell morphology and structure thus enabling to provide results for antibiotic susceptibility testing within a few days.

-Proof of concept

The proof of concept will be elaborated by exposing resistant and susceptible strains to doxycycline. Quantitative analyses will also be carried out to quantify the effects of antibiotics on *C. burnetii* growth and viability, using PCR and genome sequencing

-Efficacy assessment of different antibiotic molecules

Once the proof of concept is elaborated *Coxiella burnetiid* strains will be exposed to a panel of different antibiotics (quinolones, co-trimoxazol, macrolide…) to assess their efficacy against the bacterium.

Objet de l'étude : Evaluation of *Coxiella burnetii* antibiotic susceptibility by scanning electron microscopy

Mise en œuvre de l'étude :

This study will take place in the NSB3 laboratories and microscopy platform of the IHU (Méditerranée infection)

Echantillonnage sur le terrain:

No sampling is needed for this study.

Only collection strains provided by the national reference center (CNR) will be used.

Analyses de laboratoire :

Laboratory analysis consists on culture, electron microscopy observation and molecular biology (pcr and genome sequencing)

Objectifs primaires et secondaires :

- ✓ Development of a new antibiotic susceptibility test for *Coxiella burnetii* in axenic medium: This test will make it possible to assess *Coxiella's* response to antibiotics in axenic medium more efficiently and accurately.
- ✓ Method standardization: The aim is to establish clear protocols and guidelines to ensure reproducibility and reliability of *C. burnetii* antibiotic susceptibility testing in an axenic environment, thus facilitating comparison of results between different studies and laboratories.

Résultats envisagés :

- ✓ Standardization of a new antibiotic susceptibility testing method applied to *C. burnetii* using electron microscopy.
- ✓ Reduce the time needed to obtain *Coxiella* antibiotic susceptibility test results, from several weeks to just a few days (3 days). This will enable faster, more effective decision-making in the treatment of Q fever.

Compétences attendues :

-Experience in electron microscopy.

-Experience in bacterial culture, staining….

-Knowledge in molecular biology.

-Experience with *Coxiella burnetii* culture on axenic media is a plus.

Conditions de réalisation de la thèse :

Être titulaire d'un diplôme national de master ou d'un autre diplôme conférant le grade de master

Rémunération mensuel (151h67centième)

- 2 100 euros brut, de la date de conclusion du contrat au 31 décembre 2024 ;
- 2 200 euros brut, du 1er janvier au 31 décembre 2025 ;
- 2 300 euros brut, du 1er janvier au 31 décembre 2026.

Contact : **FOURNIER Pierre-Edouard**
E-mail : pierre-edouard.fournier@univ-amu.fr