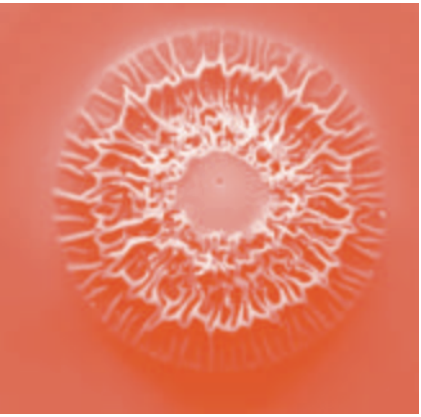
EMC 2022

European Melioidosis Congress

16-18 May | Graz, Austria



PROGRAMME & ABSTRACT BOOK

Med Uni Graz

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Welcome

Dear colleagues and friends,

On behalf of the organising committee, I would like to extend a warm welcome to you all, and hope you will enjoy what I am convinced will be an excellent European Melioidosis Congress (EMC) 2022 in Graz. As we all know, these are not easy times for travelling and attending conferences. I am, therefore, particularly pleased by the number of on-site participants from not only many different European countries but also the United States, stattdessen: Laos, Vietnam, Thailand and Nigeria, together with additional online participants, including colleagues from Bangladesh, India and Sri Lanka.

We tried to create a programme which would attract scientists from both the melioidosis and the glanders community. The scope of our programme includes clinical aspects, diagnostics, antibiotic treatment and resistance, epidemiology, genomics, pathogenesis, immune response, environmental aspects, vaccine and prevention. We hope that particularly young investigators find our congress useful and motivating. We look forward to all your contributions and the sharing of the latest knowledge over the next few days.

Since the venue is in the middle of the historical city centre, we hope you will also take the opportunity to enjoy yourself and discover some of Graz.

With best wishes,

Ivo Steinmetz, MD

Chairperson, EMC 2022 Organising Committee

General Information

Congress Chair

Ivo Steinmetz
Diagnostic and Research Institute of Hygiene, Microbiology and Environmental Medicine
Medical University of Graz

Organising Committee

David Dance
LOMWRU, Vientiane, London School of Hygiene and Tropical Medicine & University of Oxford

Susie Dunachie
MORU, Bangkok & University of Oxford

Karine Laroucau
ANSES Maisons-Alfort, Paris-Est University

Sabine Lichtenegger
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MORU, Bangkok, Mahidol University & University of Oxford

Ivo Steinmetz
Medical University of Graz

Gabriel Wagner
Medical University of Graz

Joost Wiersinga
Academic Medical Center, University of Amsterdam

Congress Venue

Congress Graz
Schmiedgasse 2, 8010 Graz

Congress Office, Registration, Sponsorship

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General Information

Registration
The registration fee includes attendance at conference sessions, booklet of abstracts, welcome reception, coffee breaks, refreshments and lunches.

Opening hours on site registration desk
Monday, May 16th 2.00-6.00 p.m.
Tuesday, May 17th from 7.30 a.m.
Wednesday, May 18th from 7.30 a.m.

Welcome Reception
Monday, May 16th 2022, 6.00 p.m.
Congress Graz (Schmiedgasse 2, 8010 Graz)

Conference Dinner
Tuesday, May 17th 2022, 7.30 p.m.
Aiola Upstairs (Schloßberg 2, 8010 Graz)

Programme Hybrid Format

SCIENTIFIC PROGRAMME
MELIOIDOSIS and GLANDERS

Monday, May 16th, 2022

2.00-6.00 p.m. Registration open
3.00-5.00 p.m. International Melioidosis Committee closed meeting
6.00 p.m. **Welcome reception**

Tuesday, May 17th, 2022

7.30 a.m. Registration open
8.30-8.45 a.m. **Opening**

8.45-10.15 a.m. CLINICAL ASPECTS & EPIDEMIOLOGY I

Invited speakers:

8.45-9.00 a.m. **Bart Currie,** Menzies School of Health Research, Darwin, Australia
Melioidosis: priority known unknowns

9.00-9.30 a.m. **Alf Fuessel,** Brussels, Belgium
Global epidemiology of glanders

9.30-10.00 a.m. **Emma Birnie**, Academic Medical Centre, University of Amsterdam, Amsterdam, Netherlands
Drivers of melioidosis endemicity: epidemiological transition, zoonosis, and climate change

Selected presentations:

10.00-10.15 a.m. **Srujana Mohanty**, All India Institute of Medical Sciences, Bhubaneswar, India
Musculoskeletal melioidosis: a single-centre experience from India over a 5-year period

10.15-10.45 a.m. Break

Programme Hybrid Format

10.45-12.30 a.m. DIAGNOSTICS, ANTIBIOTICS & TREATMENT I

Invited speakers:

10.45-11.15 a.m. **Karine Laroucau**, ANSES Maisons-Alfort, Paris-Est University, Paris, France
Diagnostic challenges in glanders

11.15-11.45 a.m. **Narisara Chantratita**, Mahidol University, Bangkok, Thailand
Identification of biomarkers for predicting mortality in melioidosis

Selected presentations:

11.45-12.00 a.m. **Mindy Elrod,** Centers for Disease Control and Prevention, Atlanta, USA
Investigation into aromatherapy spray associated outbreak of melioidosis in the United States in 2021

12.00-12.15 p.m. **Nguyen-Ho Lam**, University of Medicine and Pharmacy at Ho Chi Minh City, Ho Chi Minh, Vietnam
Antimicrobial resistance of *Burkholderia pseudomallei* causing community-acquired pneumonia

12.15-12.30 p.m. **Kay Barnes**, The Defence Science and Technology Laboratory, Salisbury, United Kingdom
Combination antibiotic therapy in a mouse model of inhalational melioidosis

12.30-1.30 p.m. Lunch

Programme Hybrid Format

1.30-3.00 p.m. CLINICAL ASPECTS & EPIDEMIOLOGY II

Invited speakers:

1.30-2.00 p.m. **Jay Gee,** Centers for Disease Control and Prevention, Atlanta, USA
Review of genomic analysis of *Burkholderia pseudomallei* associated with imports to the continental United States

2.00-2.30 p.m. **Jelmer Savelkoel**, Academic Medical Centre, University of Amsterdam, Amsterdam, Netherlands
A call to action: time to recognise melioidosis as a neglected tropical disease

2.30-3.00 p.m. **Anthony Solomon,** World Health Organization, Geneva, Switzerland
Neglected tropical diseases at WHO, and the global NTD road map 2021-2030

3.00-3.30 p.m. Break

Programme Hybrid Format

3.30-4.30 p.m. **Flash talks 1** (2 slides)
Poster presentation 1

Mégane Gasqué Anticipating emergences: spatial and molecular epidemiology of Melioidosis in French overseas departments and territories.

Sergei Biryukov Cytokine Profiles of Subunit Hcp1-based and Live Attenuated Bp 668 ΔilvI Candidate Vaccines

Sophie Guillier Characterisation of the genetic evolution of *Burkholderia thailandensis* upon antibiotic stress

Fabienne Neulat-Ripoll RNAseq analysis on *B. pseudomallei* clinical isolates and impact of phenothiazines on resistance's mechanisms

Sabine Lichtenegger *B. pseudomallei* core genome MLST analysis links melioidosis cases to a predominant genotype in a geographical *B. pseudomallei* hotspot

Sunee Chayangsu Clinical Prediction Rules for In-hospital Mortality Outcome in Melioidosis Patients

Bijayini Behera Evaluation of In Vitro Activity of Cefiderocol against *Burkholderia pseudomallei* by Broth Microdilution and Disk Diffusion

Programme Hybrid Format

4.30-6.00 p.m. DIAGNOSTICS, ANTIBIOTICS & TREATMENT II

Invited speakers:

4.30-5.00 p.m. **Herbert Schweizer**, Northern Arizona University, Flagstaff, USA
Novel insights into antibiotic resistance of *Burkholderia pseudomallei* Complex Species

5.00-5.30 p.m. **Gabriel E. Wagner,** Medical University of Graz, Graz, Austria
Point-of-care tests for melioidosis and glanders

Selected presentations:

5.30-5.45 p.m. **Adam Taylor**, The Defence Science and Technology Laboratory, Salisbury & London School of Hygiene and Tropical Medicine, London, United Kingdom
Antibody-antibiotic conjugate for melioidosis

5.45-6.00 p.m. **Riccardo D'Elia,** The Defence Science and Technology Laboratory, Salisbury, United Kingdom
Harnessing natural regulators to combat infectious diseases

7.30 p.m. Conference Dinner

Programme — Hybrid Format

Wednesday, May 18th, 2022

7.30-8.30 a.m. Registration open

8.30-9.45 a.m. PATHOGENESIS & IMMUNE RESPONSE

Invited speakers:

8.30-9.00 a.m. **Joanne Stevens**, University of Edinburgh, Edinburgh, United Kingdom
Studies on the actin-based motility and its role in the intracellular survival of *Burkholderia pseudomallei*

9.00-9.30 a.m. **Josh Hanson**, Kirby Institute, Sydney, Australia
Melioidosis of the central nervous system; impact of the bimABm allele on patient presentation and outcome

Selected presentations:

9.30-9.45 a.m. **Patpong Rongkard**, University of Oxford, United Kingdom
T-cell dysregulation is associated with fatality in community-acquired melioidosis

9.45-10.15 a.m. Break

10.15-11.45 a.m. GENOMICS & ENVIRONMENTAL ASPECTS

Invited speakers:

10.15-10.45 a.m. **Claire Chewapreecha,** Mahidol University, Bangkok, Thailand
Genome-wide association study identifies bacterial factors associated with 28-day mortality in melioidosis patients

10.45-11.15 a.m. **Rita Oladele**, Lagos University Teaching Hospital, Lagos, Nigeria.
Evaluating the presence of *Burkholderia pseudomallei* in Nigeria: an environmental soil sampling study

Programme Hybrid Format

Selected presentations:

11.15-11.30 a.m. **Karoline Assig,** Medical University of Graz, Graz, Austria
Environmental factors associated with soil prevalence of the melioidosis pathogen *Burkholderia pseudomallei:* A longitudinal seasonal study from South West India

11.30-11.45 a.m. **Apichai Tuanyok,** University of Florida, Gainesville, FL, USA
A Comprehensive Study of Prophage Islands in *Burkholderia pseudomallei* Complex (BPC)

11.45-12.45 p.m. Lunch

12.45-1.45 p.m. Flash talks 2 (2 slides)
Poster presentation 2

Hoi Cheung The role of Type VI secretion systems in infection and competition of *Burkholderia thailandensis*

Miro Plum Type VI secretion system-5 of *Burkholderia thailandensis* assembles to lyse host cell protrusions

Megan Grund BucI8-derived intranasal vaccine elicits distinct immune responses in an outbred murine model of melioidosis

Sarah Harding Modelling relapse of disease in a mouse model of melioidosis

Yuli Talyansky Host Background and Bacterial Isolate Determine Virulence and Inflammatory Response in a *Burkholderia pseudomallei* Mouse Model

Muthita Vanaporn The role of *Burkholderia pseudomallei bprE* gene in survival under stresses and infection

Programme Hybrid Format

1.45-3.30 p.m. VACCINE & PREVENTION

Invited speakers:

1.45-2.15 p.m. **Susie Dunachie,** University of Oxford, Oxford, United Kingdom
Vaccines against melioidosis: How far are we?

2.15-2.45 p.m. **Ratanaporn Tangwangvivat**, Department of Disease Control, Ministry of Public Health, Bangkok, Thailand
National plan for the prevention and control of melioidosis, Thailand

Selected presentations:

2.45-3.00 p.m. **David DeShazer,** USAMRIID, Frederick, USA
Comparison of subunit and live attenuated vaccines against aerosol challenge with *Burkholderia pseudomallei* and *B. mallei*

3.00-3.15 p.m. **Christopher Cote**, USAMRIID, Frederick, USA
Layered and integrated medical counter measures against *Burkholderia pseudomallei* infections in C57BL/6 mice

3.15-3.30 p.m. **Siobhán McClean,** University College Dublin, Dublin, Ireland
Protective BpOmpW antigen stimulates the necessary immune correlates against melioidosis

3.30-4.00 p.m. Break

4.00 p.m. **Concluding Remarks**

Announcement of WMC 2024

CLINICAL ASPECTS & EPIDEMIOLOGY I

Tuesday, May 17th, 2022

8.45-10.15 am

Invited Speakers

Melioidosis: priority known unknowns

Bart J Currie

Menzies School of Health Research and Royal Darwin Hospital, Darwin, Northern Territory, Australia

With international collaborations supporting improved microbiology capability, the true boundaries of the global presence of *Burkholderia pseudomallei* in the environment are being incrementally defined. Epidemiological and clinical studies across multiple countries have further resolved the occupational and clinical risk factors for melioidosis, the broad diversity of clinical presentations and the clinical course and outcomes with current therapy guidelines.

Some of the priority questions for further collaborative studies are:

Better definition of *B. pseudomallei* distribution across continents and within endemic countries.

How quickly is dispersal happening globally, especially in the Americas and is *B. pseudomallei* already present in the southern United States environment?

How accurate are the case numbers and mortality data predicted by modelling, especially in those countries with limited laboratory capacity and few or no cases confirmed to date?

How often does positive serology reflect latency with viable *B. pseudomallei*, rather than cleared infection (or a false +ve serology) and what is the proportion of serology positive people who eventually develop melioidosis and can this be predicted?

What is the proportion of infections from percutaneous exposure vs inhalation vs ingestion and how much does this vary by occupational, recreational, and weather parameters and how much does route of infection influence clinical presentation and outcome?

How much do the differential genomics between *B. pseudomallei* strains and the differential virulence seen in rodent models between strains of *B. pseudomallei* influence both the survival of the bacteria in different environmental niches and differences in clinical variations and outcomes in human melioidosis?

Will vaccines show benefit in human studies and if so, will they be funded for those most in need?

GLOBAL EPIDEMIOLOGY OF GLANDERS

Alf Fuessel

Glanders is a contagious and highly fatal disease affecting primarily equids. Acute forms with high fever and respiratory signs prevail in donkeys and mules. Glanders in horses generally takes a more chronic course, and asymptomatic carriers may survive for several years.

Inflammatory nodules and ulcers develop in the nasal passages and give rise to a sticky yellow discharge. The formation of nodular abscesses in the lungs is accompanied by progressive debility, coughing and possible diarrhea. Nodules are regularly found in the liver and spleen, leading to wasting and death. In the cutaneous form ('farcy'), the lymph vessels are enlarged and nodular abscesses form along their course, which then ulcerate and discharge yellow pus.

The disease, known since antiquity, is caused by the facultative intracellular pathogen *Burkholderia mallei*, a Gram-negative, non-spore forming, non-motile encapsulated rod bacterium. The most common source of infection is the ingestion of contaminated food or water, or exposure to contaminated aerosols. The bacteria can also enter the body through the contact of contaminated equipment with lesions or abrasions of the skin or through mucosa. Poor husbandry and feeding conditions and animal transport can be predisposing risk factors. For the purposes of the OIE Terrestrial Animal Health Code, the infective period of *B. mallei* in equids is lifelong and the incubation period is up to six months, but may vary depending on the route and level of exposure.

Susceptibility to the infection has also been demonstrated in camels, sheep and goats. Felines, bears and canines may become infected by ingesting the meat of infected equids. Guinea pigs and hamsters are highly susceptible and may, therefore, serve as laboratory animals ("Strauss reaction").

Glanders is a life-threatening zoonosis. Human cases have occurred through occupational contacts with infected equids and in laboratory workers. While no vaccines exist for animals and humans, human cases may be cured if treated rapidly with antibiotics. The bacterium is classified by the Centers for Disease Control and Prevention as a category B bio-threat agent.

Historically, the disease has certainly been present in all countries with a native horse population or where infected horses had been introduced. Glanders has been eradicated from many countries by the testing and destruction of infected equids and restriction on the trade in susceptible animals, not least in the context of an overall decrease of the equid population. However, it has remained endemic in certain parts of Africa, Asia, the Indian subcontinent, the Middle East and Brazil in South America, and is considered a re-emerging disease in certain previously free countries. The legal obligation to notify any suspicion of the disease, frequent testing and quarantine measures applied to the movement of horses, as well as post-mortem examination of slaughtered equids, including the splitting of heads to detect stellate scarring following upon the healing of previous ulcers, support the surveillance of the disease.

In line with the OIE Animal Health Code and following an opinion of the European Food Safety Authority, infection with *Burkholderia mallei* (glanders) is a listed disease in accordance with the European Union Regulation (EU) 2016/429 (Animal Health Law), and subject to immediate eradication (Category A) as well as trade and surveillance measures. The laboratory tests required to identify infection with *Burkholderia mallei* are those published by the EU reference laboratory for equine diseases other than African horse sickness based in Maisons-Alfort, France, in line with the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals of the OIE.

Drivers of melioidosis endemicity: epidemiological transition, zoonosis, and climate change

Emma Birnie[1,2]

[1] Amsterdam UMC, University of Amsterdam, Center for Experimental and Molecular Medicine, Amsterdam Infection and Immunity Institute, Amsterdam, Netherlands

[2] Amsterdam UMC, University of Amsterdam, Division of Infectious Diseases, Amsterdam, Netherlands

ABSTRACT

Melioidosis, caused by the soil-dwelling bacterium *Burkholderia pseudomallei*, is a tropical infection associated with high morbidity and mortality. This lecture summarizes current insights into melioidosis' endemicity, focusing on epidemiological transitions, zoonosis, and climate change. Estimates of the global burden of melioidosis affirm the significance of hot-spots in Australia and Thailand. However, it also highlights the paucity of systematic data from South Asia, The Americas, and Africa. Globally, the growing incidence of diabetes, chronic renal and (alcoholic) liver diseases further increase the susceptibility of individuals to *B. pseudomallei* infection. Recent outbreaks in non-endemic regions have further exposed the hazard from the trade of animals and products as potential reservoirs for *B. pseudomallei*. Lastly, global warming will increase precipitation, severe weather events, soil salinity and anthrosol, all associated with the occurrence of *B. pseudomallei.* Epidemiological transitions, zoonotic hazards, and climate change are all contributing to the emergence of novel melioidosis-endemic areas. The adoption of the One Health approach involving multidisciplinary collaboration is important in unraveling the real incidence of *B. pseudomallei*, as well as reducing the spread and associated mortality.

Keywords: melioidosis, *Burkholderia pseudomallei*, epidemiological transition, zoonosis, climate change, One Health

Selected presentations

Musculoskeletal melioidosis: a single-centre experience from India over a 5-year period

Mohanty S[1], *Mishra TS*[1], *Patro BP*[1], *Behera B*[1], *Purushotham P*[1]

[1]All India Institute of Medical Sciences, Bhubaneswar (AIIMS Bhubaneswar), Bhubaneswar, India

Introduction: Melioidosis, caused by the saprophytic soil pathogen *Burkholderia pseudomallei*, is increasingly being recognized in several regions of the globe, including India. The entity encompasses a variety of diverse clinical manifestations ranging from localized focal abscesses and pneumonia to sepsis with bacteremia. Recently, we have observed an increase in cases of musculoskeletal melioidosis at our institute and herein, describe the varied clinical picture of *B. pseudomallei* infection of the musculoskeletal system.

Methods: The demographic profile, risk factors and treatment outcome of patients presenting to our hospital with culture-proven *B. pseudomallei* infection of the musculoskeletal system over a 5-year period from January 2017 to December 2021 are discussed. The available medical records/charts, discharge summaries and microbiology requisition forms were reviewed for relevant clinical details and results of laboratory investigations.

Results: Of 24 patients, 17 were male and 7 female with age range 12-68 years. Septic arthritis was the commonest manifestation, followed by intramuscular abscess and psoas abscess in 10, 6 and 5 patients respectively. An underlying disorder was found in 20 (83.3%) patients. All isolates were susceptible to ceftazidime, imipenem and doxycycline, while, one isolate each was resistant to amoxicillin-clavulanate and trimethoprim-sulfamethoxazole. Twenty-one patients were surgically managed. Appropriate therapy could be provided in 22 patients with favourable outcome in 20 (90.9%), while 2 had relapses.

Conclusion: Musculoskeletal melioidosis is an emerging entity in the Eastern region of India, affecting mostly males and persons with underlying illnesses. An increasing awareness coupled with a vigilant attitude will help in early diagnosis and appropriate management.

References:

1. Perumal R, Livingston A, Samuel S, Govindaraju SK. Melioidosis of the Musculoskeletal System. Med Princ Pract. 2020;29(2):121-127. doi: 10.1159/000503021.

2. Patil HG, Gundavda M, Shetty V, Soman R, Rodriques C, Agashe VM. Musculoskeletal melioidosis: An under-diagnosed entity in developing countries. J Orthop. 2015 Sep 19;13(1):40-2. doi: 10.1016/j.jor.2015.08.001.

3. Pandey V, Rao SP, Rao S, Acharya KK, Chhabra SS. *Burkholderia pseudomallei* musculoskeletal infections (melioidosis) in India. Indian J Orthop. 2010 Apr;44(2):216-20. doi: 10.4103/0019-5413.61829.

DIAGNOSTICS, ANTIBIOTICS & TREATMENT I

Tuesday, May 17th, 2022

10.45-12.30 am

Invited Speakers

DIAGNOSTIC CHALLENGES IN GLANDERS

Karine Laroucau

Burkholderia mallei is the etiological agent of glanders, an infectious disease of equids that is gaining renewed interest due to the increasing incidence of this zoonosis worldwide. Indeed, although it was successfully eradicated in Europe and North America during the first half of the last century by slaughtering infected animals, the disease remains endemic in Africa, Asia, the Middle East and South America.

Clinical and bacteriological diagnosis in equids is particularly difficult in the early stages of the disease, when few clinical signs are expressed, and in asymptomatic carriers. Because of the low amount of bacteria present in infected tissues, abscesses, and pus, cultures are often negative, especially if samples are from subclinical or chronic cases. PCR methods have revolutionized direct diagnosis, but due to the continuous evolution of *B. mallei* strains, which are subject to numerous rearrangements, the choice of specific targets must be constantly re-evaluated.

Therefore, serology remains the method of choice for mass diagnosis. The complement fixation test (CFT) is the only test prescribed by the OIE (World Organization for Animal Health) to control the disease in international trade. This method allows the detection, even at an early stage, of asymptomatic and chronic forms of the disease, i.e. the forms most often found in animals responsible for the spread of the disease and new outbreaks. The specificity of CFT is adequate for high prevalence animal populations. It has been widely and successfully used in eradication programs. However, the CFT performance is significantly affected by the quality of the reagents used (complement, erythrocytes) and in particular by the *B. mallei* antigen applied. This method is still difficult to standardize. Alternative tests (WB, ELISA) based on bacterial suspensions or recombinant proteins have been developed. The comparison of these new tests with the reference method and their validation according to the OIE standard implies access to varied and representative panels of sera as well as to glanders standard sera.

These are the next challenges to be met in order to establish with certainty the diagnosis of glanders.

Identification of biomarkers for predicting mortality in melioidosis

Narisara Chantratita[1,2], Rungnapa Phunpang[1,2],Thatcha Yimthin[1], Taniya Kaewarpai[1], Jacqueline Margaret Cliff[3], Ji-Sook Lee[3], Clare Eckold[3], Ekkachai Thiansukhon[4], Kittisak Tanwisaid[5], Somchai Chuananont[5], Chumpol Morakot[6], Narongchai Sangsa[7], Wirayut Silakun[8], Sunee Chayangsu[9], Noppol Buasi[10], Nicholas Day[2,11], Direk Limmathurotsakul[2,12], Wasun Chantratita[15], Ganjana Lertmemongkolchai[16,17], Shelton W. Wright[18,19], T. Eoin West[1,19,20]

[1]Department of Microbiology and Immunology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand, [2] Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand, [3]Faculty of Infectious and Tropical Diseases, Department of Immunology and Infection, London School of Hygiene & Tropical Medicine, London, UK, [4]Department of Medicine, Udon Thani Hospital, Udon Thani, Thailand, [5]Department of Medicine, Nakhon Phanom Hospital, Nakhon Phanom, Thailand, [6]Department of Medicine, Mukdahan Hospital, Mukdahan, Thailand, [7]Department of Medicine, Roi Et Hospital, Roi Et, Thailand, [8]Department of Medicine, Buriram Hospital, Buriram, Thailand, [9]Department of Medicine, Surin Hospital, Surin, Thailand, [10]Department of Medicine, Sisaket Hospital, Sisaket, Thailand, [11]Centre for Tropical Medicine, Nuffield Department of Medicine, University of Oxford, Oxford, UK, [12]Department of Tropical Hygiene, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand, [15]Faculty of Medicine Ramathibodi Hospital, Center for Medical Genomics, Mahidol University, Bangkok, Thailand, [16]Cellular and Molecular Immunology Unit, Centre for Research and Development of Medical Diagnostic Laboratories (CMDL), Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen, Thailand, [17]Department of Medical Technology, Faculty of Associated Medical Science, Chiang Mai University, Chiang Mai, Thailand, [18]Division of Pediatric Critical Care Medicine, Department of Pediatrics, University of Washington, Seattle, WA, USA,[19]Division of Pulmonary, Critical Care & Sleep Medicine, Harborview Medical Center, University of Washington, Seattle, WA, USA, [20]Department of Global Health, University of Washington, Seattle, WA, USA

Abstract

Melioidosis is a severe tropical disease caused by *Burkholderia pseudomallei*. To identify biomarkers for predicting mortality in melioidosis, we characterized transcriptomes in prospectively-enrolled melioidosis patients and identified genes associated with outcome. Whole blood RNA-seq was performed in a discovery set of 29 melioidosis patients. Transcriptomic profiles of patients who did not survive to 28 days were compared with patients who survived, showing 65 genes were significantly up-regulated and 218 were down-regulated in non-survivors compared to survivors. Up-regulated genes in non-survivors were involved in myeloid leukocyte activation, Toll-like receptor cascades and reactive oxygen species metabolic processes. Down-regulated genes were hematopoietic cell lineage, adaptive immune system and lymphocyte activation pathways. RT-qPCR was performed for 28 genes in a validation set of 60 melioidosis patients, confirming differential expression between survivors and non-survivors. *IL1R2, GAS7, S100A9, IRAK3,* and *NFKBIA* were significantly higher in non-survivors compared with survivors (all $P < 0.005$). The AUROCC of these genes for mortality discrimination ranged from 0.80-0.88. In survivors, expression of *IL1R2*, *S100A9* and *IRAK3* genes decreased significantly over 28 days (all $P < 0.05$). A multi-biomarker model of plasma proteins including IL-1R2 was further developed in 78 melioidosis patients and mortality prediction was confirmed in an external validation set of 191 melioidosis patients. The biomarker model of three plasma proteins had similar or better 28-day mortality prediction compared to predictive models using clinical variables of organ failure. These data identify key pathways activated in lethal melioidosis and suggest that plasma biomarkers have potential clinical use in making decisions about triage, treatment, and referral of melioidosis patients.

Keywords: *Burkholderia pseudomallei*; RNA-sequencing; biomarkers; immune response; melioidosis; outcome; transcriptomics.

Selected presentations

Investigation into aromatherapy spray associated outbreak of melioidosis in the United States in 2021

Elrod M[1]

[1]Centers for Disease Control and Prevention, Atlanta, United States

Introduction: Between April and July of 2021, CDC was notified of four cases of non-travel associated melioidosis in four states: Kansas, Minnesota, Texas, and Georgia. All four cases were infected with the same strain of *Burkholderia pseudomallei*, identified by whole genome sequencing as likely originating from South Asia. In partnership with state and federal agencies, in depth epidemiological and laboratory investigations were conducted to determine the source of the outbreak.

Methods: Environmental and commercial product samples were collected from each of the four households. All samples were tested at CDC using RTPCR and culture, using consensus guidelines for environmental processing of samples for *B. pseudomallei* with some modifications. A total of 221 samples were tested including a variety of environmental, food, household, and personal care products. Serological testing of family members was also performed for the cases in Texas and Georgia.

Results: Serological testing results indicated exposure to a common household source, though no other family members developed disease. Sampling and testing methods were modified as the investigation progressed to enhance the odds of determining the source. Ultimately, a bottle of Better Homes and Gardens Lavender and Chamomile Essential Oil Aromatherapy room spray with gemstones from the GA patient's home was PCR and culture positive for *B. pseudomallei*. Testing of the isolate from the GA bottle indicated it genetically matched the strains that infected the four patients.

Conclusion: Testing of environmental and products samples were able to implicate the source as an aromatherapy spray. The product has been recalled by the Consumer Product Safety Commission and further investigations are underway to understand the nature of the contamination. This investigation highlights the risk of exposure to *B. pseudomallei* from imported products.

Antimicrobial resistance of *Burkholderia pseudomallei* causing community-acquired pneumonia

Nguyen-Ho L[1,2,3]*, Hoang-Thi H*[1]*, Truong-Thien P*[2]*, Le-Phuong M*[2]

[1] University of Medicine and Pharmacy at Ho Chi Minh City, Ho Chi Minh, Vietnam, [2] Cho Ray's hospital, Ho Chi Minh, Vietnam, [3] University Medical Center HCMC, Ho Chi Minh, Vietnam

Background and Objectives: *Burkholderia pseudomallei* was addressed as a pathogen causing severe community-acquired pneumonia (CAP) with a high mortality, especially in endemic areas [1]. Only a few antibiotics show efficiency in treating this pathogen. Its antimicrobial resistance has been emerging, which makes management more difficult [2]. We aim here to describe the antimicrobial resistance of *B. pseudomallei* isolated in CAP patients.

Methods: A retrospective study was conducted at the respiratory department of the largest southern tertiary hospital in Vietnam during more than two years (2020-2021). All adult patients with a diagnosis of culture-confirmed melioidosis pneumonia were enrolled.

Results: *B. pseudomallei* was isolated from specimens, such as blood, sputum, bronchial lavage and pleural pus, in 43 CAP patients (mean age 50.0; female/male 3/40). The rate of death was 23.3 %. Eight out of 43 isolates of *B. pseudomallei* showed resistance to at least one of the antibiotics currently recommended (meropenem, imipenem, ceftazidime, trimethoprim-sulfamethoxazole, amoxicillin-clavulanic). Four were resistant to meropenem, one to ceftazidime, four to trimethoprim-sulfamethoxazole, and two to amoxicillin-clavulanic. Two deaths were related to resistant *B. pseudomallei*.

Conclusion: We found *B. pseudomallei* in CAP patients that are resistant to the antibiotics which are recommended for initial intensive therapy or eradication therapy, except for imipenem.

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Combination antibiotic therapy in a mouse model of inhalational melioidosis

Barnes K[1], *Richards M*[1], *Laws T*[1], *Maishman T*[1], *Christine B*[2], *Vente A*[2], *Harding S*[1]

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Introduction: Melioidosis has a mortality rate of 10% to 50% even following antibiotic treatment, recurrence rates remain high. The investigation of different therapeutic options is important. Finafloxacin is a fluoroquinolone with improved activity in acidic conditions, and has previously shown protection against *B. pseudomallei* in murine models of infection. *In vitro* assays identified synergy between finafloxacin and doxycycline. To investigate if efficacy and bacteriological clearance could be improved *in vivo*, a combination therapy approach was investigated in a mouse model of inhalational melioidosis.

Methods: BALB/C mice were infected with *B. pseudomallei*, treatment was initiated at 24 or 36 hours post-challenge with finafloxacin (23.1 mg/kg), doxycycline (100 mg/kg) or both antibiotics in combination, by the oral route every 8 hours, and was continued for 14 days. Scheduled culls were performed at time points throughout the study, blood and organs were harvested, weighed and processed for bacterial burden. Log rank (Mantel-Cox) tests were used to analyse survival, bacterial load data was analysed using a negative binomial generalized linear model.

Results: There was no difference between treatments when initiated at 24 hours post-challenge, however there was improved bacterial control in the groups treated with the combination or finafloxacin compared to doxycycline($p<0.001$). When treatment was delayed until 36 hours post-challenge finafloxacin offered improved protection compared to doxycycline ($p<0.05$). Bacterial control with doxycycline was less successful than the combination or finafloxacin ($p<0.001$). There was also a benefit in treating with the combination compared to finafloxacin in controlling bacterial load ($p=0.006$).

Conclusion: Antibiotic combination therapy has proven to effectively offer protection, and also shows benefit in clearing infection from tissues, particularly when therapy is delayed. Further work into combination therapies is warranted.

CLINICAL ASPECTS & EPIDEMIOLOGY II

Tuesday, May 17th, 2022

1.30-3.00 pm

Invited Speakers

Review of genomic analysis of *Burkholderia pseudomallei* associated with imports to the continental United States

Gee J[1]

[1]Centers for Disease Control and Prevention (US), Atlanta, United States

Introduction: Ten to twelve cases of human melioidosis are diagnosed in the continental United States each year and are mainly associated with travel to areas where *Burkholderia pseudomallei* naturally occurs in the environment. Over the last 20 years sporadic cases have been identified in patients without a compatible travel history. These cases have been hypothesized to be caused by exposure to imported products. Also, during this time frame, imported animals such as non-human primates, iguanas, and a canine have developed melioidosis and were presumed to have been infected prior to importation.

Methods: Next generation sequencing was used to analyze single nucleotide polymorphisms (SNPs) from isolates from human and animal cases as well as from environmental samples. Genomic sequences from the cases were compared to each other as well as publicly available genome sequences.

Results: Correlations were observed for isolates from the animals and their known or putative geographic origin. Analysis of isolates obtained from recent human cases where the patients did not have a travel history indicated potential geographic origins which helped guide epidemiological investigations and environmental sampling. Analysis of isolates from environmental sampling were linked to patient isolates which demonstrated that contact with imports was the likeliest source of their infections.

Conclusion: Analysis of genomic sequences in cases without a travel history to areas endemic for melioidosis can assist in indicating the potential origin of *B. pseudomallei* which may indicate an import is involved. In cases where isolates can be recovered during environmental sampling such as from imported products, links can be established indicating the source of infection.

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Gee JE, Bower WA, Kunkel A, Petras J, Gettings J, Bye M, Firestone M, Elrod MG, Liu L, Blaney DD, Zaldivar A, Raybern C, Ahmed FS, Honza H, Stonecipher S, O'Sullivan BJ, Lynfield R, Hunter M, Brennan S, Pavlick J, Gabel J, Drenzek C, Geller R, Lee C, Ritter JM, Zaki SR, Gulvik CA, Wilson WW, Beshearse E, Currie BJ, Webb JR, Weiner ZP, Negrón ME, Hoffmaster AR. (2022) Multistate Outbreak of Melioidosis Associated with Imported Aromatherapy Spray. N Engl J Med. Mar 3;386(9):861-868.

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Gee JE, Gulvik CA, Elrod MG, Batra D, Rowe LA, Sheth M, Hoffmaster AR (2017) Phylogeography of *Burkholderia pseudomallei* Isolates, Western Hemisphere. Emerg Infect Dis. 2017 Jul;23(7):1133-1138

A call to action: time to recognise melioidosis as a neglected tropical disease

Savelkoel J[1,2], *Dance DAB*[3,4,5], *Currie BJ*[6,7], *Limmathurotsakul D*[8,9], *Wiersinga WJ*[1,2,10]

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Introduction: Despite the substantial impact of melioidosis on both the health-care systems and economies of numerous low-income and middle-income countries around the world, it is not officially classified as a neglected tropical disease (NTD) by WHO. Melioidosis causes a higher estimated disease burden and mortality than many other recognised NTDs, with deaths primarily occurring among rural poor populations in low-income and middle-income countries. We aim to present an overview of evidence in support of melioidosis meeting the criteria of inclusion as an NTD by WHO.

Methods: We gathered evidence by reviewing the literature and assessed it against the key NTD criteria. In summary, these are as follows: the disease should 1) disproportionately affect the poor and cause important morbidity and mortality 2) affect people living in (sub)tropical areas 3) be amenable to public health strategies, and 4) be relatively neglected by research.

Results: On an annual basis, modelling studies support a total of 165,000 cases worldwide of whom 89,000 die. The rural poor bear the greatest burden and it is suggested that >99% of deaths occur in low- and middle-income countries. The annual disease burden – expressed as disability-adjusted life years – is estimated at 4.64 million, higher than that of many diseases officially recognized as NTDs by WHO. Fortunately, the impact of melioidosis in a region can be reduced once awareness is established of its known or suspected endemicity.

Conclusion: The evidence gathered supports the official recognition of melioidosis as an NTD. We urge member states to request that WHO revisit their NTD list and appeal to government and philanthropic organisations in endemic countries to establish programmes to control melioidosis in order to reduce its global health burden.

References: The above abstract is an adapted version of the one as published in the Lancet Infectious Diseases. Accessible via DOI:
https://doi.org/10.1016/S1473-3099(21)00394-7.

Neglected tropical diseases at WHO, and the global NTD road map 2021-2030

Anthony Solomon

World Health Organization, Geneva, Switzerland

This presentation will briefly present the global neglected tropical disease (NTD) road map 2021-2030, and the process for considering additional diseases for the WHO NTD list.

DIAGNOSTICS, ANTIBIOTICS & TREATMENT II

Tuesday, May 17th, 2022

4.30-6.00 pm

Invited Speakers

Novel insights into antibiotic resistance of *Burkholderia pseudomallei* complex species

Herbert P. Schweizer

The Pathogens and Microbiome Institute, Northern Arizona University, Flagstaff, Arizona, U.S.A.

The *Burkholderia pseudomallei* complex (Bpc) currently encompasses eight species. Of these two cause serious diseases, *B. pseudomallei* (*Bp*) causes melioidosis and *B. mallei* (*Bm*) causes glanders. Antibiotic resistance has been mostly studied in *Bp* where resistance can occur in response to therapy and compromise melioidosis treatment. In the absence of externally acquired resistance genes *Bp*'s acquired drug resistance solely relies on its chromosomally encoded resistome. Studies focused on resistance that compromises use of antibiotics used for initial intensive melioidosis therapy (ceftazidime [CAZ] and meropenem [MEM]) and eradication therapy (trimethoprim+sulfamethoxazole [SXT], amoxicillin+clavulanic acid [AMC] or doxycycline [DOX]). The main player in CAZ^r is PenA β-lactamase where acquired resistance is the result of mutations causing: i) PenA amino acid changes, ii) *penA* overexpression by gene duplication & amplification, iii) *penA* overexpression via promoter generation & anti-termination; or iv) a combination of these mechanisms. PenA-mediated CAZ^r can be overcome by combination therapy with the β-lactamase inhibitor avibactam. CAZ^r due to penicillin-binding protein 3 target deletion has also been observed. In *Bp* and *Bm*, but not the remaining Bpc species, AMC^r is the result of a critical amino acid substitution in PenA. Studies of *penA* led to the first resistance determinant based *Bp* and *Bm* specific diagnostic. Reduced MEM susceptibility has been documented in Australian clinical isolates with either a novel PenA amino acid substitution or deregulation of the AmrAB-OprA and BpeAB-OprB efflux pumps. TMP and SXT resistance is complex and primarily due to extrusion of both drugs by the BpeEF-OprC efflux pump expressed in regulatory mutants. DOX resistance has been attributed to efflux and loss of a methyltransferase. Resistance mechanisms are conserved across Bpc species. The best studied organism aside from *Bp* is *B. thailandensis*. Summarily, acquired resistance to antibiotics in *Bp* is rare because it solely relies on mutational events affecting resident resistance determinants, rather than acquisition of external resistance genes. Understanding *Bp* antibiotic resistance mechanisms informs strategies for improved treatment of *Bp* and *Bm* infections, as well as methods for diagnosis of resistant bacteria.

Point-of-care tests for melioidosis and glanders

Gabriel E. Wagner[1], Michaela Lipp[1], Christian Kohler[2], Karoline Assig[1], Sabine Lichtenegger[1], Muhammad Saqib[3], Anne-Marie Gad[4], Ralf Ehricht[5], Karine Laroucau[6], Susanna J. Dunachie[7], Ivo Steinmetz[1]

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Background: *Burkholderia pseudomallei* and *Burkholderia mallei*, the latter a close relative that has lost its environmental reservoir and has a restricted host range, are the causative agents of melioidosis and glanders, respectively. Direct detection of both pathogens in endemic areas is often challenging and time-consuming. Therefore, there is a great need for simple, low-cost and rapid tests, which are also suitable for on-site diagnostics in rural areas. We focus here on highly standardized and sensitive serological assays that suit these requirements and, hence, might play a future role in diagnostics and epidemiological studies.

Methods: We made use of a comprehensive immunoproteomics approach in our project to identify novel *B. pseudomallei* antigens to extend our previously developed melioidosis microarray [1]. An extensive microarray screening using melioidosis and glanders sera and controls enabled the identification of promising sero-diagnostic antigen combinations, which, in turn, were used to develop point-of-care tests for melioidosis and glanders. A smartphone-based reader system enables standardized analysis and documentation of the multiplex test results.

Results: The final version of our melioidosis microarray consists of more than a hundred *B. pseudomallei* antigens, enabling comprehensive analysis of the humoral immune response in single antigen resolution. The best performing biomarker candidates from our microarray screening were tested in a point-of-care-compatible dipstick format. The 4-plex melioidosis assay achieved 92 % sensitivity and 97–100 % specificity [2], whereas the 3-plex glanders test reached 90.0 % and 100.0 %, respectively.

Conclusions: Multiplex microarray screening allows for a fast and standardized route for the guided development of sensitive and specific serological point-of-care assays [2]. The dipsticks developed benefit from their simple operation and provide rapid and accurate results. Typical issues regarding the reliable readout of highly multiplexed test can be addressed by smartphone-based detection [3]. In the future, antibody profiles might help to distinguish between glanders and melioidosis, pathogen exposure or disease amongst others. Ultimately, the toolkit developed can easily be adapted for other pathogens, rendering it a general strategy for high-resolved serosurveillance and vaccination studies, including real-time reporting if needed.

References: 1. Kohler, C.; Dunachie, S.J.; Muller, E.; Kohler, A.; Jenjaroen, K.; Teparrukkul, P.; Baier, V.; Ehricht, R.; Steinmetz, I. Rapid and sensitive multiplex detection of burkholderia pseudomallei-specific antibodies in melioidosis patients based on a protein microarray approach. PLoS Negl Trop Dis 2016, 10, e0004847.

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3. Schary, W.; Paskali, F.; Rentschler, S.; Ruppert, C.; Wagner, G.E.; Steinmetz, I.; Deigner, H.-P.; Kohl, M. Open-source, adaptable, all-in-one smartphone-based system for quantitative analysis of point-of-care diagnostics. Diagnostics 2022, 12, 589.

Selected presentations

Antibody-antibiotic conjugate for melioidosis

Taylor A[1,2], *Jenner D*[1], *Schouten J*[3], *Norville I*[1], *Prior J*[1,2,4]

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[2]London School of Hygiene and Tropical Medicine, London, Great Britain
[3]Mologic, Thurleigh, United Kingdom
[4]University of Exeter, Exeter, United Kingdom

Introduction: Melioidosis is a neglected tropical disease intrinsically resistant to antibiotics, even with prolonged antibiotic therapy there can be a relapse of infection. During infection, *Burkholderia pseudomallei* exploits an intracellular niche, avoiding extracellular antibiotic therapies. The objective of this research is to target intracellular bacteria with the use of antibodies conjugated to antibiotics. This project will develop and test an antibody-antibiotic conjugate (AAC) as a method to specifically deliver antibiotic to the intracellular site of infection.

Methods: An AAC has been designed consisting of an anti-*Burkholderia* antibody conjugated to an antibiotic via a cleavable linker. The mechanism of action relies upon opsonisation of *Burkholderia* and uptake of the AAC into phagocytes. Within the phagocyte, the linker between the antibody and antibiotic will be cleaved by cathepsin, therefore releasing the antibiotic in an active form to kill the intracellular bacteria. A macrophage infection assay is the primary method used for analysing the intracellular action of the AAC, together with confocal microscopy, imaging flow cytometry and bacterial counts.

Results: Monoclonal antibodies have been assessed *in vitro* for opsonisation ability, resulting in an anti-capsule antibody being selected for incorporation into an AAC. In addition to opsonisation, this antibody also significantly reduced bacterial actin tail formation *in vitro* [1]. Two antibiotics have been successfully chemically conjugated to a monoclonal antibody via a cathepsin cleavable linker. The AAC generated in this project, demonstrates functionality *in vitro* as a targeted delivery of antibiotic within macrophage cells.

Conclusion: This work represents an initial proof of principle in the development of an AAC as a targeted antibiotic delivery therapy for melioidosis. The targeting of antibiotic delivery is important for anti-microbial resistance concerns, limiting off target effects of the antibiotic. Targeting the intracellular site of infection has the potential to improve current antibiotic therapies for melioidosis, and reduce relapse of infection in infected individuals.

References:

[1] Taylor A, Jenner D, Rowland C, Laws T, Norville I, Prior J. Monoclonal Antibodies Opsonize *Burkholderia* spp. and Reduce Intracellular Actin Tail Formation in a Macrophage Infection Assay. J Bacteriol. 2021 Oct 12;203(21):e0024421. doi: 10.1128/JB.00244-21. Epub 2021 Aug 30. PMID: 34460311; PMCID: PMC8508110 © Crown copyright (2022), Dstl.

Harnessing natural regulators to combat infectious Diseases

Williamson ED[1], *Casulli J*[2], *Ruth H*[1], *Butcher W*[1], *Burgess G*[1], *Huxley P*[3], *Ashfield R*[3], *Travis M*[2], *D'Elia R*[1]

[1]The Defence Science and Technology Laboratory, Salisbury, United Kingdom
[2]University of Manchester, Manchester, United Kingdom
[3]Ducentis BioTherapeutics Ltd, Oxford, United Kingdom

Introduction: Traditional treatments of infectious disease have focused on the use of anti-microbial compounds (e.g. antibiotics) that target the infecting organism. However, timely diagnosis and administration of antibiotics remains crucial to ensure efficacy of these treatments especially for the highly virulent biothreat organisms (e.g. *Francisella tularensis* and *Burkholderia pseudomallei*). The need for early antibiotic treatment, combined with the increasing emergence of antibiotic resistant bacteria, means that new therapeutic strategies are required for organisms that cause rapid, acute infections.

Methods: Recent studies in our laboratories have focused on the role natural regulators (e.g. CD200, TREM1, SIRP- , mannose receptor and TAM receptors) play during infectious disease [1]. We have used *in vitro* and *in vivo* CL2/3 models of infection to determine the role natural regulators play in the disease melioidosis. Survival, bacteriological and immunological analyses have been conducted.

Results: We have demonstrated that therapeutic treatment with compounds that target the natural regulatory pathways can alter bacterial load *in vitro* and *in vivo*. In particular the use of a ligand to activate CD200R (CD200-Fc) can increase survival in a lethal *B. pseudomallei* murine model of infection when combined with antibiotics.

Conclusion: Overall, our data show that CD200R can promote antimicrobial properties and modulate the immune response and therefore may represent a novel antibacterial therapeutic target. This approach has the potential to be truly broad-spectrum, being able to treat a variety of biothreat agents and, since the strategy manipulates the host response, to be applicable not only to bacterial, but also to viral pathogens of concern.

References:

1. Casulli J, ME, Houston SA, Rossi S, Dow J, Williamson ED, Clark GC, Hussell T, D'Elia RV and Travis MA CD200R expression promotes expulsion of the bacterium *Francisella tularensis* by limiting the neutrophil niche for infection. Nature Comms 2019

PATHOGENESIS & IMMUNE RESPONSE

Wednesday, May 18th, 2022

8.30-9.45 am

Invited Speakers

Studies on the actin-based motility and its role in the intracellular survival of *Burkholderia pseudomallei*

Joanne M. Stevens

The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, UK.

The ability of *Burkholderia pseudomallei*, *B. thailandensis* and *B. mallei*, to escape into the cytoplasm of host cells and move by a process known as actin-based motility (ABM) is incredibly rare amongst intracellular bacterial species. The process involves the bacterial protein BimA and many components of the cell's own actin cytoskeleton. Early in our work on the mechanism of *B. pseudomallei* ABM, we discovered that bimA gene expression was essential for *B. pseudomallei* to move intracellularly, infect neighbouring cells, induce Multi-nucleated Giant cell formation and perhaps most importantly, survive within the infected cell. This presentation will outline our current understanding of how *B. pseudomallei* moves within the cytoplasm of the cell, and how bacterial and cellular proteins are involved in intracellular survival of this pathogenic bacterium.

Melioidosis of the central nervous system; impact of the *bimABm* allele on patient presentation and outcome

Gora H[1], *Hasan T*[2], *Smith S*[1], *Wilson I*[1], *Mayo M*[3], *Woerle C*[3], *Webb J*[3], *Currie B*[2], *Meumann E*[3], *Hanson J*[4]

[1]Cairns Hospital, Cairns, Australia, [2]Royal Darwin Hospital, Darwin, Australia, [3]Menzies School of Health Research, Darwin, Australia, [4]Kirby Institute, Sydney, Australia

Introduction: Background: The autotransporter protein *Burkholderia* intracellular motility A (BimA) facilitates the entry of *Burkholderia pseudomallei* into the central nervous system (CNS) in mouse models of melioidosis. Its role in the pathogenesis of human cases of CNS melioidosis is incompletely defined.

Methods: Methods: Consecutive culture-confirmed cases of melioidosis at two sites in tropical Australia after 1989 were reviewed. Demographic, clinical and radiological data of the patients with CNS melioidosis were recorded. The *bimA* allele (*bimABm* or *bimABp*) of the *B. pseudomallei* isolated from each patient was determined.

Results: Results: Of the 1587 cases diagnosed at the two sites during the study period, 52 (3.3%) had confirmed CNS melioidosis; 20 (38.5%) had a brain abscess, 18 (34.6%) had encephalomyelitis, 4 (7.7%) had isolated meningitis and 10 (19.2%) had extra-meningeal disease. Among the 52 patients, there were 8 (15.4%) deaths; 17/44 (38.6%) survivors had residual disability. The *bimA* allele was characterized in 47/52; 17/47 (36.2%) had the *bimABm* allele and 30 (63.8%) had the *bimABp* allele. Patients with a bimABm variant were more likely to have a predominantly neurological presentation (odds ratio (OR)

Conclusion: (95% confidence interval (CI)): 5.60 (1.52-20.61), p=0.01), to have brainstem involvement (OR (95%CI): 7.33 (1.92-27.95), p=0.004) and to have encephalomyelitis (OR (95%CI): 4.69 (1.30-16.95), p=0.02. Patients with a bimABm variant were more likely to die or have residual disability (odds ratio (95%CI): 4.88 (1.28-18.57), p=0.01). Conclusions: The *bimA* allele of *B. pseudomallei* has a significant impact on the clinical presentation and outcome of patients with CNS melioidosis.

Selected presentations

T-cell dysregulation is associated with fatality in community-acquired melioidosis

Rongkard P[1,2], *Kronsteiner-Dobramysl B*[1], *Yimthin T*[3], *Phunpang R*[3], *Dulsuk A*[3], *Hantrakun V*[2], *Wongsuvan G*[2], *Teparrukkul P*[4], *Lovelace-Macon L*[5], *Day N*[1,2], *Klenerman P*[1], *Limmathurotsakul D*[2,3], *Chantratita N*[3], *Dunachie S*[1,2], *Gharib S*[5], *West TE*[5]

[1]University of Oxford, Oxford, United Kingdom, [2]Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand, [3]Mahidol University, Bangkok, Thailand, [4]Sunprasithiprasong Hospital, Ubon Ratchathani, Thailand, [5]University of Washington, Seattle, United States

Introduction: Melioidosis is a tropical infection due to *Burkholderia pseudomallei* that commonly causes sepsis. Approximately 20% of melioidosis patients develop septic shock and estimated 50% mortality annually worldwide. Pneumonia and bacteremia are the most common manifestations of melioidosis. The high case fatality rate in resource-limited settings underscores the challenge in managing this severe infection. We aimed to identify whole blood transcriptomic signatures associated with fatality to elucidate the pathophysiology of melioidosis.

Methods: We performed whole-blood RNA sequencing on 164 melioidosis patients prospectively enrolled within 24 hours after admission to a provincial referral hospital in northeast Thailand. In addition, 25 diabetics and 25 healthy donors were recruited as uninfected controls. Subsequently, we validated our findings using an independent transcriptomic dataset derived from another acute melioidosis cohort (n=29) at other hospitals. We analyzed the associations of transcriptomic features including pathways, up-stream regulators, hub genes, and subset of immune cells with 28-day fatality.

Results: Differential gene expression analysis identified profound changes in the transcriptomic profile (633-up and 1,109-down differentially expressed genes) between non-survivors and survivors of melioidosis. Pathway analyses identified excessive inflammation and upregulated type 2 immune responses concurrent with massively down-regulated T cell mediated immunity in non-survivors compared to survivors. The transcriptomic signatures were confirmed with the validation cohort, particularly the association of elevated inflammation and reduced T-cell regulation in fatal melioidosis.

Conclusion: The early transcriptomic signature in fatal melioidosis cases is characterised by excessive inflammation, concomitant anti-inflammatory responses, and dysregulation of multiple T-cell receptor signalling. These results highlight the pivotal role of adaptive immunity during fatal melioidosis

GENOMICS & ENVIRONMENTAL ASPECTS

Wednesday, May 18th, 2022

10.15 am -11.45 pm

Invited Speakers

Genome-wide association study identifies bacterial factors associated with 28-day mortality in melioidosis patients

Claire Chewapreecha

MORU, Thailand

Melioidosis is a grossly neglected tropical disease that is still a public health burden in many low- and middle-income countries. Case fatality rates in the endemic areas range from 19-40% with diabetic patients are at risk of developing melioidosis. Currently, laboratory diagnosis of melioidosis which is largely based on the isolation of *B. pseudomallei* from clinical specimens can take up to 3-4 days. This results in delayed prescription of correct antibiotics and often patient deaths prior to the arrival of laboratory-confirmed results. To aid melioidosis treatment, we sought to identify bacterial genetic factors associated with patient 28-day mortality using the data gathered from whole-genome sequence of 1,912 bacterial isolates recovered from melioidosis patients in northeast Thailand, 28% of which were fatal cases. Using narrow-sense heritability (h2), we estimated that genomic variation in the bacteria could explain approximately 10% of mortality outcomes. Interestingly, melioidosis cases without underlying diabetes, cases with diabetes but left untreated, and cases with diabetes under antidiabetic medications displayed significantly different h2 scores. The latter had the lowest bacterial h2 and highest rates of patient survival outcomes. We thus performed genome-wide association studies (GWAS) for 28-day mortality while stratifying for patient diabetic conditions as well as bacterial population structure. Our preliminary analyses highlighted GWAS hits in 636 bacterial loci with small to moderate effect size (FDR <0.01); many of which match known bacterial virulent determinants, cell adhesins, uncharacterised proteins, and multidrug efflux pumps. The latter included AmrB operon which confers resistance to aminoglycoside and macrolide. Clinical records showed that patients were treated with a broad spectrum of Gram-negative antibiotics including aminoglycoside and macrolide in an early phase of hospitalisation before the laboratory-confirmed melioidosis results arrived. Moreover, a proportion of patients had reported self-prescription with antibiotics prior to hospitalisation. Our analyses thus linked bacterial multidrug resistance genes and a delayed in correct antibiotic treatment as one of the factors associated with 28-day mortality in melioidosis patients. We also observed improved infection outcomes in melioidosis patients with antidiabetic medication regardless of the bacterial genotypes. The use of antidiabetic such as metformin as an adjuvant to antibiotic drugs may open a new venue for treatments and is the subject of further investigation.

Evaluating the presence of *Burkholderia pseudomallei* in Nigeria: an environmental soil sampling study

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Introduction: Melioidosis, caused by the environmental pathogen *Burkholderia pseudomallei*, has been occasionally reported in southwest Africa. Other closely related *Burkholderia* species include the non-pathogenic *B. thailandensis* and *B. mallei*, the causative agent of glanders. It has been hypothesized that melioidosis is endemic in Nigeria given its favorable climate, population density and high prevalence of the main risk factor diabetes, but is underreported. We aimed to detect *B. pseudomallei* and related organisms in environmental soil samples at multiple states across Nigeria.

Methods: We performed an environmental study following consensus guidelines. Soil samples were collected from eight sites based on land use and soil type in northwestern, southwestern and southeastern Nigeria. Samples were collected at a depth of 65 cm and bacterial isolation was done using the simplified method. Presumptive *B. pseudomallei* isolates were confirmed with MALDI-TOF MS and multiplex PCR. Ten random *B. pseudomallei* isolates (i.e. one to three per site) underwent whole genome sequencing and antimicrobial susceptibility testing using the disk diffusion method following EUCAST guidelines.

Results: *B. pseudomallei* was isolated from 59 (7.4%) of 800 samples and five (62.5%) of the eight sampling sites. The maximum positivity rate for one site was 39 (39%) in Ebonyi state. In addition, we isolated *B. thailandensis* from 191 (23.9%) samples and retrieved one suspected B. mallei isolate from Ogun state in southwestern Nigeria. All *B. pseudomallei* isolates determined to be susceptible to ceftazidime, meropenem, amoxicillin-clavulanic acid, and trimethoprim-sulfamethoxazole, except one isolate showed resistance to meropenem and one showed resistance to amoxicillin-clavulanic acid.

Conclusion: We identified the environmental presence of *B. pseudomallei* in Nigeria with the highest positivity rate in the southeastern state Ebonyi. Whole genome sequence analysis is currently being performed. Future steps include a *B. pseudomallei* serological survey among residents in states with positive soil samples and a nationwide collaboration with clinical microbiology laboratories to start a prospective fever surveillance study to identify clinical cases of melioidosis in Nigeria.

Selected presentations

Environmental factors associated with soil prevalence of the melioidosis pathogen *Burkholderia pseudomallei*: A longitudinal seasonal study from South West India

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Introduction: Melioidosis is a seasonal infectious disease in tropical and subtropical areas caused by the soil bacterium *Burkholderia pseudomallei*. India is among the countries with the highest predicted melioidosis burden globally, but there is very little information on the environmental distribution of *B. pseudomallei* and its determining factors. This study aimed to investigate the prevalence of *B. pseudomallei* in soil in South West India, to determine geochemical factors associated with the presence of *B. pseudomallei* and to look for potential seasonal patterns of its soil abundance.

Methods: Environmental samplings were performed in two regions of South West India during the monsoon, post-monsoon and summer season from July 2016 to November 2018. We applied direct quantitative real time PCR (qPCR) together with culture protocols to overcome the insufficient sensitivity of solely culture-based *B. pseudomallei* detection from soil. A total of 1,704 soil samples from 20 different agricultural sites were screened for the presence of *B. pseudomallei*. Isolates were sequenced and analyzed for their core genome MLST (cgMLST) in SeqSphere (Ridom GmbH, Germany).

Results: Direct qPCR detected *B. pseudomallei* in all 20 sites and in 30.2 % (517/1,704) of all soil samples, whereas only two samples from two sites were culture-positive. *B. pseudomallei* DNA-positive samples were negatively associated with concentrations of iron, manganese and nitrogen. We found the highest number of *B. pseudomallei*-positive samples (42.6 %, $p < 0.0001$) and highest *B. pseudomallei* loads in positive samples (median 4.45 x 103 genome equivalents (GE)/g, $p < 0.0001$) during monsoon season. Values declined to 18.9 % and a median of 1.47 x 103 GE/g in summer.

Conclusion: Our study from South West India shows a wide environmental distribution of *B. pseudomallei*, but also considerable differences in the abundance between sites and within single sites. Our results support the hypothesis that nutrient-depleted habitats promote the presence of *B. pseudomallei*. Most importantly, the highest B. *pseudomallei* abundance in soil is seen during the rainy season, when melioidosis cases occur.

A Comprehensive Study of Prophage Islands in *Burkholderia pseudomallei* Complex (BPC)

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Introduction: Life cycle of *Burkholderia pseudomallei* is not entirely understood, even though it has been postulated that environmental factors could contribute to its virulence and increased disease incidences. Bacteriophages are key biological factors that influence genetic recombination and predators of *B. pseudomallei*. Phage transduction is one of the most common genetic recombination events in *B. pseudomallei*. The objective of this study was to catalog the prophages that are associated with the tRNA gene - mediated site-specific recombination or tRNA-SSR events in *B. pseudomallei* genomes.

Methods: Various bioinformatic tools were used to identify the prophages in at least 1,700 genomes of *B. pseudomallei* and other species within the *B. pseudomallei* complex (BPC). Phage induction techniques were used to propagate temperate phages in two host strains, Bp82 and 576mn, the representatives of serotype A and B, respectively. Transmission electron microscopy and whole genome sequencing were used to classify phage families.

Results: Most *B. pseudomallei* strains possess at least one functional prophage in their genomes. Ten different hotspots for phage recombination were identified, 8 of which were associated with various tRNA genes: tRNA- Phe (anticodon GAA), Met (CAT), Pro (TGG), Arg (TCT), Cys (GCA), Arg (CCG), Ser (GGA), and Sec (TCA). These tRNA-SSR events were also found in other species within the BPC. Based on the morphology and the genomic composition, there were 2 prominent phage families, Myoviridae and Siphoviridae. Multiple phage types were observed within a single strain of the BPC.

Conclusion: Most *B. pseudomallei* strains are lysogenic. Prophages are not random; most of them are associated with tRNA-SSR. We have found evidence that *B. thailandensis* temperate phages selectively infect *B. pseudomallei* hosts. This would suggest that *B. pseudomallei* populations may be controlled by the temperate phages from *B. thailandensis*, and or other BPC species in the natural environment. The interactions between the BPC species and their temperate phages warrant further investigations.

VACCINE & PREVENTION

Wednesday, May 18th, 2022

1.45-3.30 pm

Invited Speakers

Vaccines against melioidosis: How far are we?

Susanna Dunachie, Jennifer Hill, Barbara Kronsteiner, Alison Lawrie, Helen McShane, Narisara Chantratita, Julie Barbaras, Mary Burtnick, Paul Brett

Vaccination for people living in melioidosis-endemic areas has been sought for many years. A vaccine to prevent disease or death from melioidosis, targeted at people with diabetes is predicted to be a cost-effective public health intervention. The experience of COVID-19 has demonstrated how vaccines can be designed, tested and implemented – resulting in dramatic reductions in death even if "sterile immunity" preventing all infections is impossible. A desirable public health vaccine for melioidosis will be safe in immunocompromised people, elicit both antibodies and T cells, practical in terms of storage and transport, and affordable at scale in low and middle income countries. Recent excellent literature reviews have highlighted all the work on melioidosis vaccines taking place across the world, and we now benefit from a standardised mouse challenge model at USAMRID. In a collaboration between University of Nevada, Reno (UNR), DTRA and University of Oxford, we are conducting "MELVAC1" - the world's first-in-human trial of a melioidosis vaccine in Oxford, UK. We will test the vaccine candidate CPS-CRM197/Hcp1 in healthy human participants alongside people with diabetes, for safety and immunogenicity. The trial will take place in 2023 following completion of vaccine manufacture to Good Manufacturing Process (GMP). In parallel, we are undertaking a community consultation project in Ubon Ratchathani, Thailand, to explore perceptions and sensitivities for efficacy trials of a melioidosis vaccine amongst people living with diabetes and healthcare workers in Ubon.

Ultimately the melioidosis community can work together, in partnership with key stakeholders including Biodefence agencies, funder, WHO, governments and policy makers to accelerate the vaccine production pipeline. This will give a long term solution for the 280 million people with diabetes and the many with other risk factors for melioidosis including older age, chronic renal disease and immunosuppression who live in melioidosis-endemic regions in 83 countries across the globe.

National plan for the prevention and control of melioidosis, Thailand

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Melioidosis has been a neglected public health disease in Thailand. The Ministry of Public Health (MoPH) utilizes the Report 506 for the National Notifiable Diseases Surveillance System (NNDSS), and melioidosis is one of notifiable diseases in the country. Over the past 5 years (2016 – 2020), there were about 3,000 melioidosis cases per year reported to the MoPH. The total number of fatal melioidosis cases reported ranged from 14 to 233 deaths per year. Most of the reported fatal melioidosis cases were from Ubon Ratchathani province. There is an increasing evidence showing that most of the melioidosis cases reported to the MoPH are serologically diagnosed melioidosis cases, and most of the culture-confirmed melioidosis cases and fatal cases were not reported to the MoPH. Since 2020, the Department of Disease Control (DDC), MoPH has played a crucial role in reducing the burden of melioidosis in Thailand and added in the national plan for the prevention and control of melioidosis. The main objective of this program is to reduce the morbidity and mortality rate of melioidosis in Thailand. In addition, the program aims to improve the completion and accuracy of data reported to the NNDSS, and to enhance the knowledge of healthcare workers regarding melioidosis countrywide. The launched program consists of 1) a revision of the definition of melioidosis case needed to report the NNDSS 2) the online training for healthcare workers in human, animal and environmental sectors 3) enhancing the investigation report 4) enhancing the reporting systems 5) strengthening melioidosis networks 6) sharing information among sectors 7) raising awareness in risk groups. The program has been receiving good attention from public health officers, healthcare workers, village health volunteers and people in the country. Extra-fund will be allocated to implement the national programme at target provinces. Monitoring and evaluation framework will be applied to assess an achievement of program and identify gaps and challenges for program improvement.

Key words: Melioidosis, morbidity, mortality, plan, surveillance, Thailand

Selected presentations

Comparison of subunit and live attenuated vaccines against aerosol challenge with *Burkholderia pseudomallei* and *B. mallei*

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Introduction: *Burkholderia pseudomallei* is a Gram-negative pathogen and agent of melioidosis, a disease ranging from acute and rapidly fatal to protracted and chronic. The closely related species, *B. mallei*, is an obligate pathogen of mammals including solipeds and humans and causes glanders, a disease with manifestations similar to those of melioidosis. No vaccines have achieved total protection against long-term infection or consistently provide complete sterilizing immunity. We evaluated subunit and live attenuated vaccines for their ability to protect mice against *B. pseudomallei* and *B. mallei* strains.

Methods: C57BL/6 mice were administered the live attenuated strain *B. pseudomallei* 668 ilvI on days 0 and 24. Mice were also administered three doses of two subunit vaccine candidates, composed of Hcp1 and capsule conjugated to CRM197 (conjugate) +/- AhpC + alhydrogel and CpG. Submandibular blood collections were taken approximately 1 week prior to challenge in order to assess the resulting humoral immune response by ELISA. Approximately one month after the last vaccine, the mice were exposed to aerosolized *B. pseudomallei* K96243 or *B. mallei* FMH and monitored for sixty days.

Results: Following challenge with 4 LD50 of K96243, mice vaccinated with conjugate + Hcp1, 668 ∅ilvI, or conjugate + Hcp1 + AhpC exhibited 80%, 60%, or 50% survival, respectively. Mice were also vaccinated with conjugate + Hcp1 or 668 ∅ilvI and exposed to 4 LD50 and 11 LD50 of *B. mallei* FMH in two separate aerosol challenges of ten mice per group (mean 7.5 LD50). While only 25% of the adjuvant control mice survived for 60 days post challenge, the conjugate + Hcp1 and 668 ∅ilvI vaccinated mice exhibited survival rates of 80% and 85%, respectively.

Conclusion: The subunit and live attenuated vaccines provided similar protection against *B. pseudomallei* and *B. mallei*, but they generated distinct humoral and cellular immune responses. The data suggest that conjugate + Hcp1 and 668 ilvI are strong candidates for ongoing vaccine development against both melioidosis and glanders. Future vaccination strategies may benefit from heterologous priming schedules in which the second vaccine dose uses a different vaccine than the first dose.

Layered and integrated medical counter measures against *Burkholderia pseudomallei* infections in C57BL/6 mice

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Introduction: *Burkholderia pseudomallei*, the gram-negative bacterium which is the causative agent of melioidosis, is notoriously difficult to treat with antibiotics. A significant effort has focused on identifying protective vaccine strategies. However, when used as individual medical countermeasures both antibiotic treatment plans (therapeutics or post-exposure prophylaxes) and vaccine strategies remain suboptimal. Our objective was to improve overall disease outcome by layering vaccines with antibiotic regimens.

Methods: C57BL/6 mice that were naïve or vaccinated with two doses of the live attenuated vaccine strain 668 ∅ilvI or three doses of the recombinant protein Hcp1 +/- AhpC + capsule polysaccharide conjugated to CRM197+alhydrogel + CpG ODN2006 were exposed to aerosolized *B. pseudomallei* K96243 approximately 30 days after the last vaccination. Co-trimoxazole was then initiated as early as 9 h or as late as 117 h post-exposure to *B. pseudomallei*. The antibiotic regimens consisted of dosing every 12h (Q12) for either seven or 21 days. Mice were observed for at least 60 days post-cessation of antibiotics.

Results: When used in combination, current vaccine strategies (recombinant protein subunits Hcp1 +/- AhpC plus capsular polysaccharide conjugated to CRM197 or the live attenuated vaccine strain 668 ∅ilvI) and co-trimoxazole regimens result in near uniform protection in the mouse model of melioidosis due to synergy associated with the medical counter measures. These results were similar when examining several sub-optimal antibiotic regimens (e.g. seven day antibiotic course initiated early after infection or 21 day antibiotic course with delayed initiation).

Conclusion: Layered and integrated medical countermeasures will provide novel treatments to combat *B. pseudomallei* infections but also diseases caused by other bacterial pathogens that are refractory to individual strategies, particularly in the case of engineered or emerging bacterial biothreat agents.

Protective BpOmpW antigen stimulates the necessary immune correlates against melioidosis

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Introduction: There is a serious unmet clinical need for a vaccine to protect people against melioidosis. In particular, people with diabetes mellitus are most at risk of melioidosis, with a 12-fold increased susceptibility for severe disease. We previously identified *B. pseudomallei* OmpW (BpOmpW antigen) as a potential vaccine candidate. Immunisation with this antigen protected C57BL/6 mice from lethal *B. pseudomallei* challenge for up to 81 days [1]. In this study we aimed to elucidate the immune correlates of protection of the protective BpOmpW vaccine as an essential step prior to clinical trials.

Methods: BpOmpW was expressed, purified and endotoxin levels measured. An insulin-resistant (IR) mouse model was developed by feeding on a high fat diet (12 wk), monitoring insulin resistance and weight gain. Non-IR or IR C57BL/6J mice were immunised with BpOmpW and recall responses in splenocytes determined using an antibody panel specific for CD4, CD49b, CD45RB, CD8a, CD25, CD44, CD3 surface markers and intracellular IL-2, IL-4, IFN- , IL-17, IL-9, TNF- and FoxP3. Recall responses in HLA transgenic mice, proliferation of human PBMCs in response to antigen and serology were also determined.

Results: BpOmpW induced strong antibody responses, stimulated effector CD4+ and CD8+ T cells and CD4+ CD25+ Foxp3+ Treg cells, and produced high IFN- responses in CD4+, CD8+, NK, and NKT cells in non-IR mice. T-cell responses of IR mice to BpOmpW were comparable to those of non-IR mice. Humanized HLA-DR and HLA-DQ transgenic mice elicited IFN- recall responses in ELISpot analyses. Moreover, human donor peripheral blood mononuclear cells (PBMCs) exposed to BpOmpW for 7 days showed T-cell proliferation. Finally, plasma from melioidosis survivors with diabetes recognized the BpOmpW vaccine.

Conclusion: The immune response to BpOmpW in IR mice was generally comparable to that observed in non-IR mice, indicating the antigen will be protective in the diabetes context. The strong recall responses of HLA transgenic mice to BpOmpW and the T-cell proliferation observed in human PBMCs strongly indicate that BpOmpW is immunogenic for T-cell responses in humans. Overall, the range of approaches used show that BpOmpW elicits the necessary immune responses to combat melioidosis and bring this vaccine closer to clinical trials.

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POSTER

1 Clinical aspects

1.1
Clinical Prediction Rules for In-hospital Mortality Outcome in Melioidosis Patients

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Introduction: Melioidosis is an infectious disease caused by *Burkholderia pseudomallei*. It is an endemic disease in tropical areas. Although, there are effective treatment, but the mortality rate is still high. There were many risk factors associated mortality. This study aims to develop a scoring system for predicting the in-hospital fatal melioidosis using various factors.

Methods: The data were collected from Surin hospital, Surin, Thailand, between April 2014 and March 2017. We included patients older than 15 years, confirmed by culture positive for *Burkholderia pseudomallei*. The clinical prediction rules were developed using significant risk factors from the multivariable analysis

Results: In the final analysis model, 251 patients were used for identifying the significant risk factors of fatal melioidosis. Five factors were identified and used for developing the clinical prediction rules, and the factors were as follows: qSOFA≥2 (odds ratio [OR]=2.39, P=0.025), abnormal CXR finding (OR=5.86, P<0.001), Cr≥1.5mg/dL (OR=2.80, P=0.004), AST≥50U/L (OR=4.03, P<0.001), and CO2≤20meq/L (OR=2.96, P=0.002). The prediction scores ranged from 0 to 6.5. A high-risk group (3.5-6.50) predicted a high mortality rate>76.1%(LR+=3.74). A low-risk group predicted a lower mortality rate (LR+ 0.35) and AuROC 84.6%.

Conclusion: This scoring guideline was used to predict the higher in-hospital fatal melioidosis. The usefulness of these prediction scores is for guiding to closely monitor and provide aggressive treatment in high-risk group.

2 Antibiotic Treatment and Resistance

2.1
Modelling relapse of disease in a mouse model of melioidosis

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Introduction: *Burkholderia pseudomallei* is resistant to many antimicrobial agents, most apparent in acidic conditions that may be encountered in vivo. New antibiotics are being evaluated, as monotherapies and combination therapies. Finafloxacin has previously shown protection against *B. pseudomallei* in murine models of infection. In an attempt to improve efficacy (and reduce relapse of infection) a combination therapy approach was investigated and statistics performed on different data sets generated to determine whether relapse of disease and bacterial colonisation could be predicted.

Methods: BALB/C mice were infected with *B. pseudomallei* and treated from 24 or 36 h post-challenge, with finafloxacin, doxycycline or both antibiotics. Mice were weighed and observed for clinical signs of disease. A number of scheduled culls were performed and organs processed for bacterial burden. Logrank (Mantel-Cox) tests were used to compare the time to death to the development of three criteria: a clinical score of 2 or more for 2 days, 10% weight loss or weight loss on 3 days totalling 5%. The relationship between the measurable time to colonisation and time to the above was investigated.

Results: There is a strong correlation between the time to the development of a clinical score of 2 or more for 2 days, 10% weight loss or weight loss for 3 days totalling 5% and time to death, particularly for the animals treated from 36 hours post-challenge. The colonisation data suggests that the majority of the animals treated from 36 hours that recorded one of the 3 parameters (as above) but did not succumb to infection, would have died/been culled. The average time from the 'parameter being recorded to observed bacterial colonisation was 2-8 days.

Conclusion: Further studies will use this modelling and statistical tools to investigate observed versus estimated relapse and colonisation.

2.2
Evaluation of *In Vitro* Activity of Cefiderocol against *Burkholderia pseudomallei* by Broth Microdilution and Disk Diffusion

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Introduction: Cefiderocol, a novel siderophore cephalosporin, is in spotlight for treatment of difficult to treat Gram negative bacilli infections with limited treatment options. With expanding burden of melioidosis, the in vitro activity of new antimicrobials against clinical *B. pseudomallei* isolates needs to be ascertained. This study was conducted to determine the in vitro activity of Cefiderocol against a contemporary collection of *B. pseudomallei* isolates by the reference broth microdilution method (BMD). Performance of disk diffusion (DD) was evaluated as an alternative convenient testing method.

Methods: A total of 60 *B. pseudomallei* isolates from culture confirmed melioidosis patients in a tertiary care hospital of Odisha, between 2018-21, were included. Cefiderocol minimum inhibitory concentration (MIC) was determined by using Iron depleted cation adjusted Mueller-Hinton broth (ID-CAMHB) as per Clinical and Laboratory Standards Institute (CLSI) recommendations. DD was performed on unsupplemented Mueller-Hinton agar using 30µg Cefiderocol disc. Susceptibility patterns were interpreted as per CLSI M100 S31. *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used as control strains for BMD & DD

Results: All isolates were susceptible to Cefiderocol based on CLSI breakpoints of Cefiderocol against Pseudomonas aeruginosa, *Acinetobacter baumannii*. Cefiderocol MIC range of 60 *B. pseudomallei* isolates were 0.125 to 2 mg/liter. Both MIC50, and MIC90 were 0.5mg/litre.Zone diameters ranged from 31 to 40 mm.

TABLE 1. In vitro MICs ranges for 60 *B. pseudomallei* isolates against Cefiderocol

***B. pseudeomallei* (n=60)**	**No. of isolates at Cefiderocol MIC (mg/liter)**							
	0.125	0.25	0.5	1	2	4	8	16
	5	23	26	4	2	0	0	0

Conclusion: Cefiderocol exhibited excellent in vitro activity against 60 isolates of *B. pseudomallei* from Odisha, India.The MIC90 of the 60 isolates in our study was 0.5 mg/litre, slightly higher than MIC90 values of 0.125 mg/litre of 246 clinical isolates of *Burkholderia pseudomallei* from Queensland, Australia [1] and MIC90 of 0.25 mg/litre obtained from a study of 30 USAMRIID *B. pseudomallei* isolates [2]. Relationship between Cefiderocol zone diameter and MIC values can be better ascertained by including resistant isolates. Further clinical trials are needed to prove clinical efficacy of Cefiderocol

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2.3
RNAseq analysis on *B. pseudomallei* clinical isolates and impact of phenothiazines on resistance's mechanisms

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Introduction: *Burkholderia pseudomallei* possess many intrinsically resistances to various antibiotics. Active pumps efflux is one of the main mechanisms implicated in Multi-Drug Resistant phenotype, and 3 main RND efflux pumps have been described. As bacteria can become drug resistant by overexpressing their efflux systems, new strategies have been developed by targeting these pumps in order to restore the initial susceptibility to antibiotics. Our laboratory chose to design phenothiazine derivatives molecules (efflux pumps inhibitors) for enhancing the efficiency of existing antibiotics.

Methods: Our laboratory chose to design phenothiazine derivatives molecules (efflux pumps inhibitors) for enhancing the efficiency of existing antibiotics (1). In a previous study, we screened 47 molecules on *Burkholderia thailandensis*, a study model of *B. pseudomallei*. Effects were observed with several molecules by determining Minimal Inhibitory Concentration (MIC) of different antibiotics. Thus, two candidate molecules, AST2 and AT17 were identified and further tests conducted with *B. pseudomallei* collection strains. Here, we propose to test these two molecules on clinical strain *B. pseudomallei*

Results: Despite being isolated from the same patient, *B. pseudomallei* A2-B1-C strains present natural differences on antibiotic resistance. Genomic analysis by WGS did not allow us to highlight genes implicated in resistance mechanisms, and the natural resistance of B1 et C strains is transiant, with a diminution of the resistance MIC after 5 or 10 subcultured. Phenothiazines have 2 main effects: AST17 restore the antibiotic sensibility and AST2 has antagonistic effect with an augmentation of the resistance. RNAseq analysis were performed with or without molecules on *B. pseudomallei* strains.

Conclusion: Here, RNAseq analysis performed on the BP strains with or without molecules reveal genes which are implicated in the mechanisms of antibiotic resistance. In the future, bioinformatic integrative analysis with transcritomics, protomics, phenotypics data will allow us to better understand the complex mechanisms involved in the resistance.

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2.4
Characterisation of the genetic evolution of *Burkholderia thailandensis* upon antibiotic stress

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Introduction: *Burkholderia pseudomallei*, the causative agent of melioidosis, is considered as a potential bioweapon due to its natural resistance to a large panel of antibiotics. This feature limits the therapeutic arsenal to a few antibiotics. Difficulties in diagnosis and long treatment are the main causes of relapse and the emergence of new clinical resistances. In this project, we were interested in determining the genetic evolution of *Burkholderia thailandensis*, considered as an alternative model of *Burkholderia pseudomallei*, when stressed with antibiotic.

Methods: *Burkholderia thailandensis* E264 strain was subjected to increasing concentrations of trimethoprim-sulfamethoxazole, with successive passages on agar and liquid medium. This antibiotic is indicated during the eradication phase of melioidosis. We decided to sequence the cultivated population to mimic what happens during infection with evolution within host. The whole genome sequencing of stressed populations was performed with Illumina technology. By using the computational tool BRESEQ [1], we identified and calculated the proportion of SNPs/INDELs in all generated populations.

Results: Under low antibiotic pressure, several mutations occurred in the efflux pump regulatory genes (BpeS, BpeR, BpeT). Some were associated with multidrug resistance patterns in intermediate concentrations but disappeared at highest concentration, while those specific to trimethoprim-sulfamethoxazole became fixed and dominant. Concomitantly, the acquisition of mutations in the folate pathway genes (FolA and FolM) was progressive. It became exclusive at the highest antibiotic concentration and confer resistance to Trimethoprim-Sulfamethoxazole.

Conclusion: Some of these genetic variations obtained are already described in the literature in *Burkholderia pseudomallei*, giving consistency to our model. Others identified mutations are unpublished to our knowledge [2,3]. This methodology seems to be very interesting for the identification of mutations in efflux pump regulatory genes that could appear very early in the process of acquisition of antibiotic resistance observed in certain clinical strains. They would play the role of an early biomarker, helping the clinician in the choice of antibiotic treatment.

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3 Epidemiology

3.1
B. pseudomallei core genome MLST analysis links melioidosis cases to a predominant genotype in a geographical *B. pseudomallei* hotspot

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Background: Four epidemiologically linked melioidosis cases were reported recently from a small geographical area in Vietnam. Targeted environmental sampling revealed a high environmental *B. pseudomallei* prevalence where the cases occurred. We present here an extended genomic and phenotypic characterisation of environmental and clinical strains.

Methods: Environmental strains from the epidemiologically linked rice and sugar cane field, an open drainage of the street and a nearby streamline were isolated using our recently published two-step method [1]. A cgMLST analysis was performed on all isolated strains, since this method offers a fast and simple high-resolution whole genome sequence analysis [2]. Finally, we examined selected *B. pseudomallei* isolates in our human macrophage-based infection model [3].

Results: The cgMLST-based genotyping revealed a genetically diverse population consisting of 19 different complex types with one predominant environmental genotype in 31 out of 61 isolates. The same genotype was also isolated from three of the four patients. Preliminary data also show that strains belonging to the environmentally and clinically dominant genotype exhibit increased intracellular bacterial loads in human macrophages compared to other environmental isolates.

Conclusions: *B. pseudomallei* is genetically diverse in agricultural land in Vietnam, although a predominant genotype was detected in a geographical hotspot. Our results indicate a possible superior environmental fitness and virulence of this genotype. We will perform subsequent sampling of the respective fields and in-depth virulence analyses to confirm this hypothesis. Based on the genetic heterogeneity observed in the fields, we suggest tight sampling strategies to determine sources of infection. Furthermore, this study demonstrates the potential of *B. pseudomallei* cgMLST for genomic epidemiology.

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4 Pathogenesis

4.1
Type VI secretion system-5 of *Burkholderia thailandensis* assembles to lyse host cell protrusions

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Introduction: During intracellular cell-cell spread, *Burkholderia pseudomallei* uses actin-tails to form protrusions into neighboring host cells1,2. The type VI secretion system-5 (T6SS-5) is a main virulence factor required for efficient spread of *B. pseudomallei* between host cells3. Previous studies have shown that T6SS-5 is involved in inducing multi-nucleated giant cells and in lysing host cell-cell protrusions4. However, the underlying mechanism and the spatiotemporal regulation of assembly of this secretion system is unclear.

Methods: In our study, we visualized the assembly and firing of the T6SS-5 in live host cells via confocal microscopy. Moreover, we genetically engineered different *Burkholderia thailandensis* T6SS-5 mutant strains to evaluate their spreading behavior inside the host cells.

Results: In our study, we visualized the assembly and firing of the T6SS-5 in live host cells via confocal microscopy. Moreover, we genetically engineered different *Burkholderia thailandensis* T6SS-5 mutant strains to evaluate their spreading behavior inside the host cells.

Conclusion: We were able to visualize T6SS-5 assemblies in live host cells and could directly show how the T6SS-5 lyses host cell-cell protrusions. Our data could also provide insights into the host cell-cell spread of *B. pseudomallei* and enable refining diagnostic approaches and development of potent antimicrobials.

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4.2
Host Background and Bacterial Isolate Determine Virulence and Inflammatory Response in a *Burkholderia pseudomallei* Mouse Model

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Introduction: *Burkholderia pseudomallei* (Bp) infection onset, duration, and severity are highly variable and its mechanisms of pathogenicity as well as host risk factors remain poorly understood. After infecting BALB/C and C57BL/6 mice with a panel of Bp strains with variable median lethal doses (LD50) between 1.0x10^1 and 4.2x10^4 CFUs [1], we measured bacterial burden and host innate immune response over time in order to identify how these factors affect disease outcome.

Methods: BALB/C and C57BL/6 mice were infected intraperitoneally with Bp strains 1106a, HBPUB10134a, and MSHR5855 as well as the *Burkholderia mallei* (Bm) strain FMH. Bacterial burdens in the blood and spleen were measured via dilution plating and host gene expression in whole blood was analyzed via microarray. RAW264.7 murine macrophage-like cells were infected with Bp strains followed by measurement of host cell death pathway genes and bacterial T3SS and T6SS genes via RT-qPCR. Previously banked formalin-fixed, paraffin-embedded splenic tissues were analyzed for caspase-3 activation via IHC.

Results: In BALB/C mice, bacterial splenic burden remained high when infected with two out of three strains. Genes involved in pyroptosis, apoptosis, and necroptosis were expressed in blood in the first two days post-infection with MSHR5855 but not 1106a. Immunohistochemistry of infected spleens revealed caspase-3 activation differed by strain. Analysis of Bp genomes revealed variable tracts of short simple repeats directly preceding the Type 6 secretion system-regulating *bprC* gene, and significant variation in *bprC* expression between strains correlated with host cell death transcription *in vitro*.

Conclusion: Significant variation in virulence, bacterial splenic burden, and induction of host inflammatory and cell death pathways exists among Bp strains. We hypothesize these significant variations may be, in part, mediated by differential activation of the T6SS driven by the expansion of short simple repeats in the intergenic region directly preceding the *bprC* regulator gene in the T3SS locus.

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4.3
The role of *Burkholderia pseudomallei bprE* gene in survival under stresses and infection

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Introduction: *Burkholderia pseudomallei* can survive for years in the environment and persists under various stress conditions. Our previous study demonstrated that sigma E (*rpoE* gene) the first gene in *B. pseudomallei RpoE* operon (*rpoE, bprE, rseB*, and *mucD*) has important roles in stress response, intracellular survival, and the regulation of *rpoH* expression. The second downstream gene, BPSL2435 (*bprE*), a putative sigma E negative regulator (anti- E), would also play an important role. The objective is to determine the role of *bprE* gene in bacterial survival under stresses and infection.

Methods: The *bprE* mutant and complementary strains were constructed using *B. pseudomallei* 576 strain as a wild-type. Their survival under oxidative stress (H_2O_2, t-BOOH, and menadione), salt stress (2 M NaCl) and high temperature (50°C) were compared and analyzed. The biofilm formation and the intracellular survival within macrophage cell line J774A.1 were determined.

Results: The bprE mutant exhibited high sensitivity to H_2O_2, t-BOOH, menadione, 2 M NaCl and high temperature (50°C) and also reduced the percentage of survival in macrophage J774A.1 cell lines with statistical significance ($P < 0.05$). But biofilm formation was not significant difference when compared between each strains.

Conclusion: Our data suggests that *bprE* might plays a role in sigma factor E negative regulation and bacterial survival under stress conditions. The sigma factors competition and *RpoE* expression level during stress induced may explain the loss of ability to survive under stress conditions in *bprE* mutant which would be clarified in further study.

4.4

The role of Type VI secretion systems in infection and competition of *Burkholderia thailandensis*

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Introduction: Type VI Secretion System (T6SS) is used by wide range of bacteria to deliver toxins into neighbouring cells and thus T6SS plays an important role during bacterial competition as well as host infection. *Burkholderia* species contains multiple T6SS, which can target eukaryotic or bacterial cells or have other functions. The factors influencing the dynamics of the T6SS during infection and bacterial competition are yet to be fully understood. We aim to explore the intracellular and environmental signals that regulate T6SS and how it affects bacterial spread and pathogenesis.

Methods: To investigate the regulations of T6SS, targeted mutagenesis, infection assays of different host cell types and live-cell imaging were performed on *B. thailandensis*.

Results: We observed that bacterial signalling affects the dynamics of the anti-eukaryotic T6SS-5. The lack of certain signalling molecules leads to a "hyper-firing" phenotype, and it contributes to an increased formation of multi-nucleated giant cells. Moreover, we found that the anti-bacterial T6SS is expressed during infection but there are unknown factors that block its assembly.

Conclusion: In summary, the anti-eukaryotic and anti-bacterial T6SS of *B. thailandensis* have different regulation mechanisms. Understanding the signals for regulation allows us to gain a better understanding on the spread of *B. thailandensis*.

5 Immune Response

5.1
Cytokine Profiles of Subunit Hcp1-based and Live Attenuated Bp 668 ΔilvI Candidate Vaccines

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Introduction: *Burkholderia pseudomallei* (Bp) is naturally resistant to various antibiotics which results in a high mortality rate, thereby necessitating for a safe and effective vaccine. The immune response in C57BL/6 mice was evaluated pre- and post-aerosol challenge with Bp K96243 after vaccination with live attenuated Bp 668 ΔilvI strain. Also, we tested two candidate subunit vaccines, composed of Hemolysin co-regulated protein 1 (Hcp1) mixed with the capsule polysaccharide conjugated to the highly immunogenic CRM197 protein carrier (Conjugate) with and without alkyl hydroperoxide reductase (AhpC).

Methods: The C57BL/6 mice were administered three doses (days 0/21/38) of the subunit vaccines (Hcp1 + Conjugate or Hcp1 + Conjugate + AhpC) and two doses (days 0/24) of Bp 668 ΔilvI were given. The mice were exposed to approximately 1.35 x 10^3 CFU (~3.4 LD50) of aerosolized Bp K96243 at 38 days after the last vaccination. Tissues and blood were collected for immunoassays at three intervals: 6 and 27 days after vaccination and 3 days after challenge (day 79) for cytokine and bacteriological analyses.

Results: Six days post-vaccination the number of IFN-y secreting splenocytes were enumerated after stimulation with purified Hcp1 or AhpC antigens. Stimulation with Hcp1 and AhpC significantly upregulated IFN-y secreting splenocytes in the Conjugate + Hcp1 and Conjugate + Hcp1 + AhpC vaccine groups, respectively. Three days post-challenge with Bp K96243, all vaccine groups displayed a significant reduction in cytokine levels compared to the controls, in the lung homogenates, with the exception of elevated INF- and IL-22, specifically in the Bp 668 ΔilvI vaccinated group.

Conclusion: The overall suppression (or reduced upregulation) of cytokine/chemokine production, such as IL-1 , IL-1β, IL-6, TNF- , IL-18, CXCL1, and CCL3 in vaccine compared to control groups, suggests that the vaccines protected the mice against the destructive effects of hypercytokinemia. The unique cytokine profiles of these three protective vaccines suggests that each vaccine may promote distinct mechanisms of protection. Heterologous vaccination regiments that combine subunit and live attenuated candidate vaccines may further augment the protective efficacy against melioidosis.

6 Environmental Aspects (*B. pseudomallei*)

6.1
Anticipating emergences: spatial and molecular epidemiology of Melioidosis in French overseas departments and territories.

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Introduction: The presence of autochthonous human cases of melioidosis in the French overseas territories raises the question of *Burkholderia pseudomallei* presence locally. The aim of our project is to determine the spatial occurrence of *B. pseudomallei* in these territories in order to define disease risk areas. As large-scale environmental surveys are complex, we performed serological tests in a panel of domestic animals with the hypothesis that seropositive animals could be indicative of the presence of *B. pseudomallei* in their immediate environment and thus target areas for environmental investigations.

Methods: Blood samples were collected in farms during routine testing or at slaughter in French Guiana, Martinique and Guadeloupe. The study was conducted on 1 435 animals including cattle, goats, sheep, pigs and canines. Sera were analysed with the commercial GLANDA ELISA kit (IDvet, France) developed for the diagnosis of glanders in equids and based on a recombinant protein of B. mallei that has been shown to cross react with *B. pseudomallei*. This method does not require a specific conjugate, and the criterion established for glanders was used for our analysis.

Results: In French Guiana, the analysis of samples from cattle (18), sheep (10) and goats (6) farms revealed seropositive animals in 4 (24 positive animals /154), 2 (4/85) and 1 (1/61) farms, respectively. In Guadeloupe, the analysis of 3 goat farms revealed seropositive animals in the 2 commercial farms corresponding to 14 out of 31 goats. In Martinique, the analysis of sera collected in slaughterhouses (305 cattle, 89 goats and 177 pigs) and in a dog rescue center (74) revealed positivity for 4 cattle, 4 goats and 1 pig. None of the tested animals showed clinical signs.

Conclusion: In endemic areas, the interpretation of the serological results can be hindered by the cross-reactivity with *B. thailandensis* or the inability to differentiate infection from background antibody levels. In non- endemic areas, serological screening could help to identify exposed animals and to target specific areas for the search of the bacterium. In our study, we identified seropositive animals in French overseas territories. Next, we will conduct local environmental studies to confirm the presence of the bacterium and ultimately confirm the pertinence of this ELISA kit for screening.

7 Vaccine and Prevention

7.1
Bucl8-derived intranasal vaccine elicits distinct immune responses in an outbred murine model of melioidosis

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Introduction: *Burkholderia pseudomallei* and *B. mallei*, respectively the causative agents of melioidosis and glanders, are infectious species of both clinical and biodefense concerns. Currently there is no licensed vaccine. Here, we developed an outbred model of melioidosis to assess vaccines formulated with antigens derived from an outer membrane protein, *Burkholderia* collagen-like protein 8, or Bucl8. Bucl8 has a homotrimeric structure that includes a beta-barrel with two surface-exposed loops per monomer, designated L1 and L2, that are conserved between *B. pseudomallei, B. mallei*, and *B. thailandensis*.

Methods: Two peptides encompassing L1 and L2, which epitopes were predicted to be immunogenic, non-toxic, and non-allergenic, were selected as a basis for the vaccine. Outbred CD-1 mice were immunized intranasally with vaccine formulations—composed of loop-peptides conjugated to diphtheria toxoid CRM197 and adjuvants—to assess the antigen-specific immune responses in mouse sera and lymphoid organs. Immunity to Bucl8 antigens was also assessed following natural infection with luminescent *B. thailandensis* E264, as a model of respiratory melioidosis.

Results: Antigen combined with the adjuvant F-CDG elicited significantly increased levels of antigen-specific mucosal IgA and systemic IgG than an LPS-based adjuvant. IgG and IgA were present in the nasal wash, saliva, and sera two weeks post-immunization. Inoculation with an LD50 dose of bacteria resulted in acute respiratory tract infection over the course of four days. Mice exhibiting high luminescent signal and increased bacterial burden in the lungs progressed to a moribund stage. Mice that survived the infection decreased or cleared nasal and lung colonization by day 7.

Conclusion: In conclusion, we optimized a vaccine formulation to elicit antigen-specific mucosal responses and we developed an outbred model of respiratory melioidosis using B. thailandensis, a previously established surrogate to *B. pseudomallei*, for future assessment of protection by Bucl8-based subunit vaccine.

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