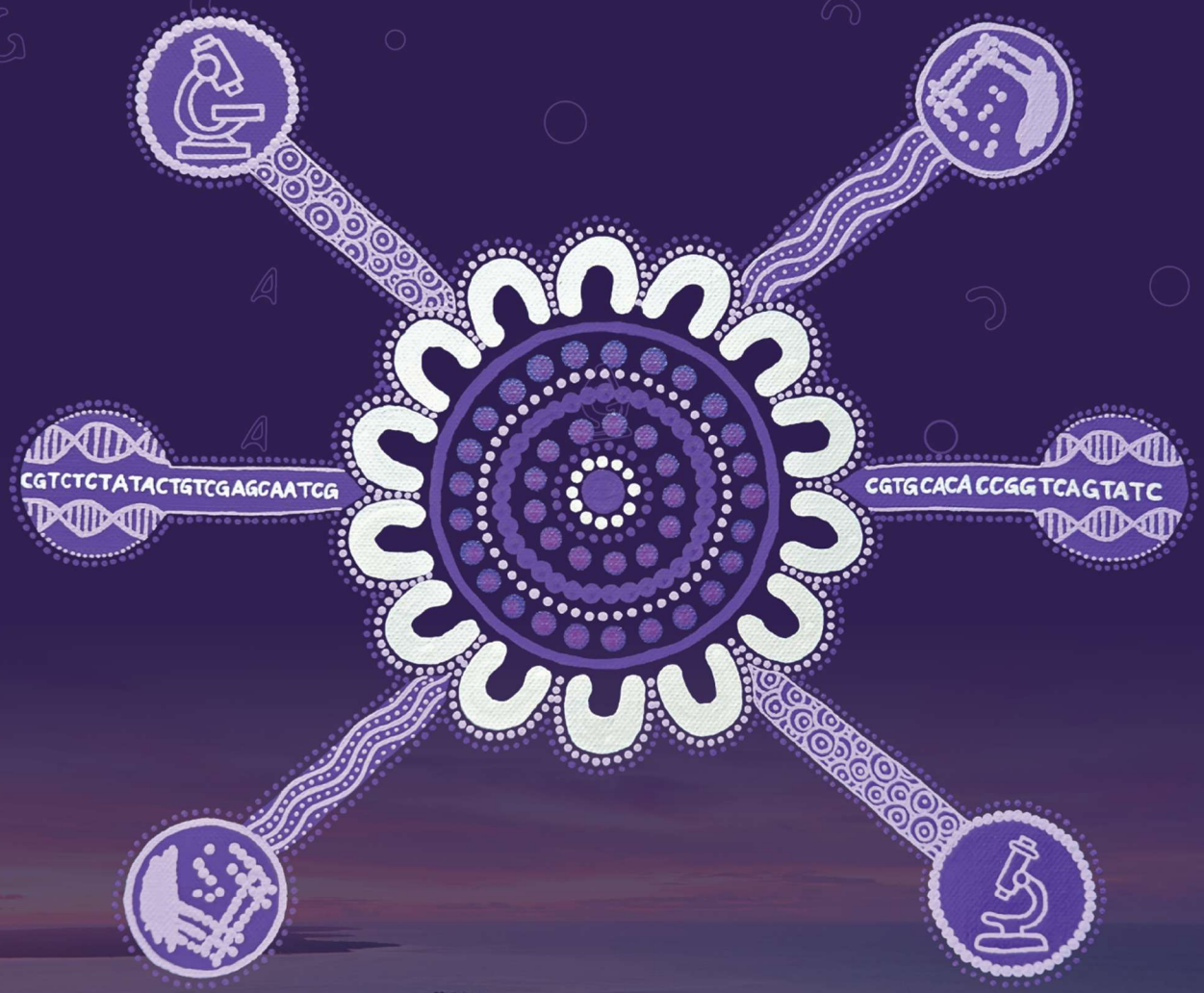
menzies
school of health research

POSTER ABSTRACTS



10TH WORLD MELIOIDOSIS CONGRESS

21-23 October 2024

10th World Melioidosis Congress
Darwin, Northern Territory, Australia
21-23 October 2024

Poster Abstracts

Veterinary Cases of Melioidosis at Ocean Park in Hong Kong: 1998 – 2023

Chan, San Yuen; Kwok, Kelly; Ho, Hok Lai; Hui, Suk Wai; Martelli, Paolo

Veterinary Centre, Ocean Park Corporation, Hong Kong, SAR China

Burkholderia pseudomallei bacterium is the causative agent of melioidosis in humans and animals. A retrospective review of 52 veterinary melioidosis cases at Ocean Park during 1998–2023 involved captive marine mammals (13 cetaceans and 18 pinnipeds) and 21 birds. The study linked melioidosis prevalence to inclement weather, with 36 culture-confirmed and 16 presumptive diagnoses.

Seroprevalence, determined by ELISA, was 0.15% for cetaceans, 1.81% for pinnipeds, and 1.92% for birds using microagglutination test. Multilocus sequence-typing (MLST) of *B. pseudomallei* isolates from environmental and clinical samples revealed that ST70 was the predominant strain, followed by ST37, ST32, ST660 and ST684, linking the source of infection to the animal environment. Two novel strains, ST660 and ST684 were first identified. Cases correlated positively with typhoon periods ($P<0.005$), average monthly rainfall ($P=0.005$), and environmental *B. pseudomallei* isolates from soil/rainwater ($P=0.005$).

The disease was diagnosed sporadically over 18 of 26 years, with 96% of cases occurring from April to October. Timeline showed 10 cases (1998–2001), 15 outbreak cases in 2002, 5 cases (2003–2006), and 19 cases within (2007–2010, 2016, 2021), with at most 1 case reported in 2012, 2013, and 2015.

Mortality rates were reported as 19.2% among marine mammals and 30.8% among birds, with no mortalities in either group in the past 15 and 7 years, respectively. Melioidosis (cases/year) decreased from 4.5 (1998–2003) to 1.9 (2004–2013) and further to 0.6 (2014–2023). The decline in cases was attributed to advancements in diagnostics, treatments, behavioral training, and husbandry, including animal facilities.

Animal Melioidosis in the Northern Territory: Summary of Submissions to Berrimah Veterinary Laboratory

Shilton, Cathy; Benedict, Suresh.

Berrimah Veterinary Laboratory, Darwin, NT, Australia

Over 35 years, *Burkholderia pseudomallei* was isolated from 260 laboratory submissions. Diagnosis was through bacteriological isolation techniques using sheep blood agar, MacConkey agar, Ashdown media and phenotypic speciation. The majority of cases (71%) were identified during the rainy season (November – April). Among domesticated mammals, goats accounted for 40% of the cases, followed by pigs (13%), dogs (9%), sheep (7%), and cats (7%). Despite occurring in large numbers in the NT, melioidosis has never been diagnosed in cattle and in only one horse. Melioidosis in poultry is rare; most avian cases occurred in captive parrots. Crocodiles appear to be resistant-melioidosis has only been diagnosed in two farmed hatchlings. Differential pathology suggests various modes of transmission and pathogeneses. Goats present with various distinct syndromes, including fatal haemorrhage resulting from dissecting aneurysms due to suppurative vasculitis, cranial nerve deficits due to suppurative encephalitis involving the brainstem, hind limb paralysis due to lumbar vertebral osteomyelitis with extension to the spinal canal and dyspnoea due to respiratory infection. These presentations are often accompanied by abscesses in the spleen and/or liver and/or terminal embolic showering of the lung. In pigs, abscesses of the spleen and/or liver are the most common presentation. In dogs, the main pathology in necropsy submissions was pyogranulomatous brainstem encephalitis while samples taken from live dogs suggest a predilection for cutaneous and urinary tract infection. In parrots, the usual presentation was severe disseminated abscessation involving the spleen, liver and lung.

Seroprevalence of Melioidosis and its Associated Risk Factors – A Population-Based Study in Odisha, Eastern India

Behera, Bijayini; Singh, Arvind K; Ahmad, Mohammad; Rout, Lipipuspa; Jena, Jayanti; Patnaik, Asmita; Behera, Pradeep; Priyadarshini, Payal; Pandey, Dhruv; Chan, Po-Lin; Dutta, Biswa Prakash; Thapa, Badri; Pritam, Jitendriya Amrit; Mohanty, Srujana; Mahapatra, Ashoka; Mishra, Abhishek; Mohapatra,Prasanta Raghab;

All India Institute of Medical Sciences [AIIMS], Bhubaneswar, Odisha.751019, India

Melioidosis, caused by *Burkholderia pseudomallei*, is an emerging disease in Odisha, a state in eastern coastal India. Difficult to diagnose, the disease is likely to be severely underreported. Seroprevalence studies in the general population are deemed necessary for an estimate of melioidosis endemicity as well as to explore the associated risk factors. A population-based cross-sectional seroprevalence study was conducted using Indirect Haemagglutination Assay (IHA) among 1920 participants aged 5-60 years residing in six out of thirty districts of Odisha from August to December 2023. Seropositivity was defined as an IHA titre ≥ 1:20. The risk factors associated with seropositivity were determined. Out of 1920 individuals, 1215 (63.3%) were females and 1680 (87.5%) were rural residents. 410 out of 1920 individuals had IHA titre ≥ 1:20, contributing to an overall prevalence of 20.9% [95% CI: 19.0% - 22.7%]. Seropositivity was highest among those aged 21 to 30 years [23.2% (95% CI: 19.2% - 27.4%)], and in females [21.7% (95% CI: 19.4% – 24.1%)]. The odds of seropositivity was 1.64 [95% CI: 1.097 - 2.436, p= 0.016] times higher among rural residents compared to urban residents. The present study, covering a single post- monsoon season in Odisha, have yielded a 20.9% melioidosis seropositivity, matching previous Indian studies with *B. pseudomallei* seropositivity rates ranging from 20-29%. The study indicates towards widespread environmental presence of B. *pseudeomallei*, more so in rural areas and thus risk of clinical melioidosis. Adoption of public health interventions as well creation of awareness is of paramount importance in such scenario.

Seroprevalence of Antibodies to *Burkholderia pseudomallei* in Gulf Coast, Mississippi, September 2023

DeBord, Katherine M; Elrod, Mindy; Hartloge, Claire; Meyer III, William A; Swanson, Brooke E; Schrodt, Caroline A; Negron, Maria E; Weiner, Zachary P.

Division of High Consequence Pathogens and Pathology, Centers for Disease Control and Prevention, Atlanta, GA

In 2022, *Burkholderia pseudomallei* was first identified in the continental United States (US) in environmental samples from the Mississippi Gulf Coast. To better understand the extent of exposure to this emerging bacterium in the US, we tested a convenience sample of 825 residual sera samples (550 from the Mississippi Gulf Coast, 275 from the northern US) collected in September 2023 by a commercial diagnostic laboratory for the presence of antibodies to *B. pseudomallei*, using an indirect hemagglutination assay (IHA). We estimated seroprevalence of antibodies to *B. pseudomallei* in the Mississippi Gulf Coast region and compared it to the seroprevalence seen in northern regions of the US, which are less likely to support persistence of *B. pseudomallei* in the environment. Overall, 52% (432/825) of specimens were from female patients and the median patient age was 57 (IQR: 37, 66) years with no differences between Mississippi Gulf Coast and control residents. Using a serology cutoff of 1:40, 14% (78/550) of specimens from Mississippi Gulf Coast residents, 17% (47/275) from control residents, and 15% (125/825) overall were positive for *B. pseudomallei* antibodies. Similarities in seropositivity between Mississippi Gulf Coast residents and northern US residents suggest environmental exposure to *B. pseudomallei* in the Mississippi Gulf Coast may be limited; however, lack of accompanying illness and exposure information limits our ability to conclusively interpret these findings. These estimates can serve as a baseline of seropositivity in the US for future studies and to track the spread of *B. pseudomallei* in the US over time.

Burkholderia pseudomallei, the causative agent of melioidosis, is locally established in Guadeloupe, French West Indies

Gasqué, Mégane; Guernier-Cambert, Vanina[1,2]; Terret, Jules; Aaziz, Rachid; Manuel, Gil; Breurec, Sébastien; Rochelle-Newall, Emma; Laroucau, Karine

[1]Sorbonne University, IRD, INRAÉ, CNRS, UPEC, UMR iEES-Paris, 75005 Paris, France; [2]Faculty of Veterinary Technology, Kasetsart University, Bangkok, Thailand

Since 1993, 21 human melioidosis cases have been reported in Martinique and Guadeloupe (French West Indies, FWI), raising the question of importation *versus* autochthonous transmission. Our study aimed to confirm the endemicity of *B. pseudomallei* in the FWI and French Guiana using a One Health approach. We conducted serological and environmental surveys to assess animals' past exposure to *B. pseudomallei* and try to isolate the bacterium.

Blood samples were collected from cattle, goats, dogs, equines and pigs in the FWI and French Guiana, and rectal swabs from goats. Soil and water samples were collected from selected farms. Animal exposure to *B. pseudomallei* was assessed by serology using an ELISA kit, and the presence of the bacteria in feces and the environment was tested by culture and targeted PCR.

The serological survey identified seropositive animals in all areas, with high seroprevalence in cattle (45/347, 12%), equines (46/295, 15%) and goats (12/31, 37%). Further investigations were carried out in two goat farms in Guadeloupe. PCR-positive rectal swabs and environmental samples were identified in these farms, and *B. pseudomallei* was successfully isolated from a soil sample in Guadeloupe, confirming the local presence of *B. pseudomallei*. Molecular analyses revealed a high degree of similarity between the soil-isolated strain and those isolated from patients in the FWI. Our serological results, combined with comprehensive environmental surveys, can help identifying high–risk areas for investigation, as well as disseminating information to the local population. Environmental surveys will now be extended to Martinique and French Guiana.

Melioidosis in central India: A hitherto naïve agricultural state with a significant burden of the disease

Gupta, Ayush[1]; Dadheech, Malti[1]; Siddiqui, Farha[1]; Purwar, Shashank[1]; Malhotra, Anvita Gupta [2]; Singh, Jitendra[2]

[1]Dept. of Microbiology, All India Institute of Medical Sciences (AIIMS) Bhopal; [2]Dept. of Translational Medicine, AIIMS Bhopal

India is predicted to have the highest burden of Melioidosis in the world however, it remains grossly underdiagnosed with only coastal states of southern & eastern India namely, Karnataka, Tamil Nadu, Kerala and Orissa regularly reporting the disease. Post-COVID pandemic there is a surge in the cases being diagnosed in other parts of the country. We report a series of cases diagnosed in the state of Madhya Pradesh (MP), a predominantly agricultural, landlocked state in central India. Before this, there were reports of a few cases (≤5) belonging to MP diagnosed outside the state. Between 2020 - 2022, we diagnosed 51 cases of melioidosis in our institute of which major clinical manifestations were bone & joint infections (29%), pneumonia (25%), skin and soft tissue infections (23%), and acute febrile illness without evident foci (14%). The detailed demographic description of the cases is provided in Table 1. The district-wise geographical location of these cases where the details could be retrieved is provided in Figure 1. We found that these patients resided in 14 different districts indicating that melioidosis is endemic throughout the state of MP but grossly underdiagnosed. We performed Whole genome sequencing of 6 clinical isolates using the Ion GeneStudioTM platform. MLST assignments of 6 isolates using the PubMLST database showed that these isolates belonged to 5 STs namely 43, 659, 859 (2), 1427 and 1555 which have been previously reported from southern India.

Two Decades of Melioidosis in India: A Comprehensive Epidemiological Review

Kaur, Harpreet[1]; Kannan, Sriram[1] and Mukhopadhyay, Chiranjay[2]

[1]Indian Council of Medical Research Hqrs., New Delhi, India; [2]Department of Microbiology, Kasturba Medical College, Manipal Academy of Higher Education, Manipal, India

Melioidosis is a neglected tropical disease with lethal outcomes in patients with diabetes or any other chronic illnesses like kidney or liver disease. The comparison of 4 sets of 5-year literature in last 2 decades showed that the number of publications, predominantly on clinical- epidemiological studies was highest from the Southern India followed by the Eastern, with an increasing trend from other parts especially during 2019-2024. Nine studies during 2010-2022 found fever accounting for 86% (SD 12%) cases, followed by cough and joint pain (26%, SD 17%) and (23%, SD 21%) respectively. While the predisposing conditions included diabetes (75%, SD 9%), alcohol abuse (19%, SD 9%) and cancer (6%, SD 1%), the clinical presentations included bacteremia (50%, SD 38%), pneumonia (37%, SD 23%), splenic abscess (18%, SD 16%) and skin & soft tissue infection (16%, SD 10%). Further, using the diabetes and poverty hotspots, melioidosis was predicted to be prevalent in northeast India that had least no of publications but reported many conducive factors for melioidosis. Studies have further observed people with risk factors like diabetes or chronic kidney diseases while working outdoors or indoors in varied professions who are more prone to have melioidosis. The awareness on melioidosis is increasing in India as observed by publications from different parts in the last 5 years with an overall importance being on clinical epidemiology studies, and less on anti-microbials, vaccines, diagnostics and environmental surveillance. The identification of hotspots could be done using diabetes hotspots and the areas marked below poverty line.

Burkholderia pseudomallei seroreactivity amongst febrile hospital patients in Northern Vietnam identifies spatio-ecological signatures of disease and reveals genetic near-neighbor infections

Metrailer, Morgan C.; Tran, Thi Le Quyen; Pham, Van Khang; Luong, Tan Minh; Jiranantasak, Treenate; Bluhm, Andrew P.; Hoang, Thi Thu Ha; Luong, Minh Hoa; Do, Bich Ngoc; Pham, Thanh Hai; Norris, Michael H.; Trinh, Thanh Trung; Blackburn, Jason K.

Spatial Epidemiology and Ecology Research Lab, Department of Geography, and the Emerging Pathogens Institute, University of Florida, Gainesville, Florida, United States of America

In Vietnam, melioidosis is not a nationally reportable disease and little is known regarding the epidemiology of melioidosis in northern regions despite endemicity and high hospital burden described in the central region. This study targeted six northern provinces of Vietnam: Son La, Dien Bien, Lai Chau, Lao Cai, Ha Giang, and Cao Bang. The objective of this study was to 1) elucidate the distribution, spatio-ecological signatures, and seasonal patterns of melioidosis in Northern Vietnam, and 2) use whole genome analysis to inform on isolated species in suspected seropositive patients. ELISA assays specific to *Burkholderia pseudomallei* were used to determine melioidosis seroprevalence from febrile provincial hospital patients with unknown causes of disease (2020-2023). Case data were aggregated to patient home communes for spatial Bayes rate smoothing and spatial clustering with local Moran's I. A presence/absence analysis was performed to elucidate the relationship between environmental/physical conditions and melioidosis presence. Blood culturing and WGS of a subset of positive patient samples was performed. We identified urban hotspots and a correlation between hospital proximity and melioidosis positive samples, suggesting that commune-level health campaigns could enhance melioidosis surveillance regionally. Improved surveillance in rural communes is also suggested as hotspots of melioidosis seroprevalence were identified in rural regions, despite limited sampling. Seropositivity was related to soil conditions associated with *B. pseudomallei* presence and land use. Seasonality and melioidosis seropositivity was established, with the wet season having high absorbance. Phylogenetic analysis revealed genetic near-neighbor species to *B. pseudomallei*, all of which persist in similar environments.

Melioidosis in France over the last twelve years

Ombeline Lamer, Flora Nolent, Olivier Gorgé, Fabienne Neulat-Ripoll

French Medical Research Institut (IRBA), Bacteriology Unit, Brétigny-sur-Orge, France

Melioidosis is endemic in South East Asia and Northern Australia, but recent publications highlighted a wider distribution of Burkholderia pseudomallei, the bacterium responsible for the disease. Since its extended maritime territory, France, through its overseas territories, falls into the area of presence of *B. pseudomallei*. In historical records, most French melioidosis cases were imported from South East Asia. However, the improvement of diagnostic tools, especially Mass Spectrometry, has led to an increase of cases, not only in Indian Ocean, but also in West Indies. Over the last twelve years, we have participated to the analysis of 79 cases, mostly from South East Asia, with an increase of Indian ocean cases.

Here we review the geographical origins of all cases diagnosed and their specificities like antibiotic profiles and host susceptibility and MLST analysis.

Expanding the molecular epidemiology of melioidosis in North Central Vietnam

Norris, Michael H.[6], Metrailer, Morgan C.[1,2], Nguyen, Viet Ha[3], Tran, Thi Le Quyen[3], Jiranantasak, Treenate[1,2], Bluhm, Andrew P.[1,2], La, Thi Hai Au[6] Hoang, Thi Thu Ha[4], Hoa, Luong Minh[4], Pham, Thanh Hai[4], Bui, Nguyen Hai Linh[3], Hang, Nguyen Thi Thu[5], Trinh, Thanh Trung[3], Blackburn, Jason K.[1,2]

[1]Spatial Epidemiology & Ecology Research Laboratory, Department of Geography, University of Florida, Gainesville, Florida, United States of America; [2]Emerging Pathogens Institute, University of Florida, Gainesville, Florida, United States of America; [3]Institute for Microbiology and Biotechnology, Vietnam National University, Hanoi, Vietnam; [4]National Institute of Hygiene and Epidemiology, Hanoi, Vietnam; [5]National Institute of Veterinary Research, Hanoi, Vietnam; [6]Pathogen Analysis and Translational Health Group, School of Life Sciences, University of Hawai'i at Mānoa, Honolulu, Hawai'i, United States of America

Melioidosis cases have been recorded for decades in Vietnam. The 9th World Melioidosis Congress was held in Hanoi and perception of the public health impact increased. Melioidosis is not nationally reportable in Vietnam. More research is needed to understand disease ecology and public health impacts in the country. Data on strain characteristics and case epidemiology are needed to estimate propensity of *Burkholderia pseudomallei* genotypes to transmit from soil to humans. Thirty-five *B. pseudomallei* clinical isolates from provincial hospitals, ten from soil, one from swine, and one from a bear were collected and sequenced by Illumina. Clinical strains were isolated from patients aged from 26 to 82 from Ha Tinh province in each month of 2020 (except July). Four of the clinical isolates were new sequence types (ST) as determined by traditional seven marker MLST analysis. Twenty of the thirty-five (57%) clinical strains isolated in this study were ST 41and obtained across the year. The earliest ST 41 isolates recorded were from a human infection in the USA (1983) and a human infection in Vietnam (1998) indicating ST 41 has a long history and is an important epidemiological ST in Ha Tinh. cgMLST identified finer scale spatial differences. ST 41 was recovered from one soil sample ~1 year after clinical isolates. The remaining soil isolates did not match clinical STs; one matched an ST from a swine in Nghe An to the north. As melioidosis moves towards reportable in Vietnam, molecular epidemiological methods can connect human, veterinary, and environmental genotypes.

MELIOIDOSIS: AN UNTOLD STORY FROM NORTH-EAST OF INDIA

Phukan Chimanjita; Khandait Ranjita

Department of Microbiology, Assam Medical College & Hospital, Dibrugarh; Srimanta Sankaradeva University of Health Sciences, Assam, India

Abstract: Melioidosis, a life-threatening neglected infectious disease caused by Burkholderia pseudomallei, is endemic to certain tropical and subtropical regions and may be acquired through haematogenous spread, inhalation or skin penetration. The diversity of clinical presentation varies and the risk for disease severity increases in patients with diabetes mellitus, chronic renal insufficiency, underlying lung disease and alcohol abuse. Our study presents a case series of patients presenting with soft tissue infection of the left lower eyelid near the inner canthus, non-healing lesions in foot and leg and multiple abscesses and systemic involvement. The cases with skin and soft tissue infections had a common history of working in the paddy fields and showed gradual improvement with treatment. Melioidosis has been diagnosed in immuno-compromised patients, who presented with splenic abscess and pleural effusion in a sickle cell anaemia patient, urinary incontinence with prostatic enlargement and multiple abscesses with hepatosplenomegaly in patients with uncontrolled diabetes mellitus**.** The commonly associated risk factors were diabetes (33.33%) and alcohol consumption (83.33%). Melioidosis was more prevalent in the middle age group with a male-to-female ratio of 2:1 and half of the population being tea garden workers. Amongst these cases, mortality was 16.67%. Although Burkholderia is intrinsically resistant to gentamicin, two cases (33.33%) showed the presence of gentamicin-sensitive strains, confirmed by the MIC broth dilution method, requiring further molecular evaluation and sequencing. The present scenario indicates that north-eastern India might be an endemic hub for melioidosis where cases are being neglected and under-diagnosed necessitating better diagnostic strategies and awareness.

Descriptive study on epidemiology, demographic variation and occupational exposure among culture positive melioidosis patients from a tertiary care hospital in southern Sri Lanka.

Piyasiri, Bhagya; Jayasekera, Krishantha; Corea, Enoka

National Hospital Galle, Karapitiya, Sri Lanka

Melioidosis is a multi-system disease among at-risk population, caused by the soil bacterium *Burkholderia pseudomallei*. Here, an anonymous database of 90 culture positive melioidosis patients from December 2014 to July 2024 in the largest tertiary care hospital of southern Sri Lanka was accessed and analyzed to observe the demographic variation and the occupational exposure. There were 68 (75%) males and predominantly 41-60 years age group was affected (48, 53%). Almost all patients were from the 3 districts of the Southern province except 3. Exposure to soil was admitted by 57 (63%) by being a farmer, estate or manual worker (16, 20), gardening (13), or playing in mud (2). There were 4 fishermen, 4 retired soldiers who fought in the war front >10 years back, 4 tsunami victims in 2004, and 12 flood victims. There were 8 tractor or lorry drivers involved in sand loading and transport of soil contaminated goods. Two chefs were there who denied any sort of exposure to soil or contaminated water. Two got the disease after near drowning. Prevalence of diabetes and chronic kidney disease was 73% (66/90) and 17% (15/90) respectively in this cohort. Alcoholism and smoking were noted in 30 (33%). Mortality was 19% (17/90) and 4 died on the consecutive 2nd admission before any diagnosis. Sixty-nine (77%) were discharged to the clinic follow-up.

Conclusions: Melioidosis was more prevalent in the working men with exposure to soil and contaminated water. Majority were diabetics. More awareness is needed for early diagnosis on clinical grounds.

Endemicity, Travel, Outbreaks, and Genomics: Understanding Melioidosis Surveillance in the United States, 2008 – 2022

Richardson Jr., Brian T.; DeBord, Katherine M.; Schrodt, Caroline A.; Petras, Julia K.; Cossaboom, Caitlin M.; Elrod, Mindy G.; Gee, Jay E.; Gulvik, Christopher A; Weiner, Zachary P.; Bower, William A.; Hoffmaster, Alex R; Negron, Maria E.

Bacterial Special Pathogens Branch, Division of High-Consequence Pathogens and Pathology, Centers for Disease Control and Prevention, United States of America

Melioidosis, a disease caused by *Burkolderia pseudomallei,* has historically been thought of as a tropical and subtropical disease. In 2023, melioidosis became nationally notifiable in the United States (U.S.) due in part to the expanding endemic distribution. In 2022, locally-acquired melioidosis was identified in the southern U.S. This finding suggest melioidosis is newly endemic in the U.S. We described melioidosis epidemiology in the U.S., including case numbers, risk factors, exposures, spatial distribution, past outbreaks, and advancements in genomic analysis. From 2008-2022, the CDC received 104 melioidosis case reports from 35 jurisdictions. Among cases with data, 82% (n=85) had documented international travel; 64% (n=66), reported travel to a melioidosis endemic region. Sixty-eight percent of cases (n=70) were hospitalized; 9% (n=9) died. In the 30 days prior to illness onset, 12% (n=13) of cases reported contact with animals; 21% (n=22) reported contact with freshwater or mud. Seventy-five percent (n=78) of cases reported preexisting conditions, with diabetes being the most common (34%). Twelve cases (11%) had likely or confirmed domestic exposure. Genomic analysis and epidemiologic data linked 4 cases to imported contaminated aromatherapy spray from India and one case to a freshwater home aquarium containing imported tropical fish. From 2020-2023, three local cases were linked to *B. pseudomallei* isolated from soil and water samples in Mississippi in 2022. This report emphasizes the increasing presence of melioidosis in the U.S. Additionally, it highlights the importance of making melioidosis nationally notifiable, enhanced surveillance, timely diagnostics, and advancements in genomic analysis to better understand melioidosis epidemiology.

Serological evidence of exposure to *Burkholderia pseudomallei* in Nigeria

Savelkoel, Jelmer[1]; Wagner, Gabriel E; Ojide, Chiedozie K; Lipp, Michaela; Frankenfeld, Katrin; Rudloff, Anne; Dunachie, Susanna J; Wiersinga, W Joost; Steinmetz, Ivo; Birnie, Emma; Oladele, Rita O

[1]Amsterdam UMC location University of Amsterdam, Amsterdam, the Netherlands

Background

Melioidosis is being reported on the African continent with increasing frequency. In Nigeria, the highest soil positivity rate of its causative agent *Burkholderia pseudomallei* has been established in the southeastern state of Ebonyi. However, epidemiological data remain limited. This study aims to determine the extent to which soil positivity elicits a serological response in inhabitants exposed to *B. pseudomallei*.

Methods

We performed a cross-sectional study to assess seropositivity by recruiting healthy participants in Ebonyi state. Serum and information on *B. pseudomallei* exposure risk was obtained. We screened serum on a multiplex immunoassay for the presence of antibodies using the *B. pseudomallei* antigens BPSS0477, BPSL2096, BPSL2697 and BPSS1498. Seropositivity was defined as a response to ≥1 antigen. Serum from healthy subjects from highly endemic Ubon Ratchathani, Thailand was included as a comparator.

Results

In this cohort of 500 healthy participants (predominantly males with a median age of 30 years), we observed an overall seropositivity rate of 30% compared to 46% in the Thai cohort (n = 50). Using logistic regression with an overall seropositivity rate as outcome, we did not find significant predictors of a positive serological response, which included barefoot soil exposure and farming.

Conclusions

We provide the first rough seropositivity estimates of exposure to *B. pseudomallei* in Nigeria, which can be used to explore predictors for seropositivity in the Nigerian population and for future serosurveillance efforts and modelling studies. Our multiplex immunoassay should be validated in larger cohort studies in endemic areas to determine its potential and the extent of possible cross-reactivity.

A hidden threat: First evidence of *Burkholderia pseudomallei* silent exposure in Madagascar

Razafimahatratra, Solohery Lalaina[1]; Rajerison, Mino[1]; Phillips, Carina[2]; Wagner, David[2]; Settles, Erik[2]; Schoenhals, Matthieu[1]

[1]Institut Pasteur de Madagascar, Madagascar; [2]Northern Arizona University, USA

B. pseudomallei has been isolated from clinical and environmental samples near Mahajanga in Madagascar, indicating the disease's endemic presence. However, the overall distribution and prevalence of *B. pseudomallei* and melioidosis in Madagascar is unknown.

Using a Luminex xMAP® assay to detect antibodies specific to 6 highly reactive *B. pseudomallei* antigens in culture-confirmed melioidosis individuals' serum samples (GroEL, AhpC, HCP1, CPS, LPSA, LPSB), we screened 5,738 serum samples collected since 2020 in five Malagasy regional blood transfusion centers. A threshold of the mean standardized MFI plus two standard deviations was set, and statistical analysis was performed.

Higher reactivity to HCP1 and CPS was found in samples from Mahajanga. Two individuals from Mahajanga (n=2/1009) tested positive for all six antigens. Interestingly both these individuals were young male field workers. Statistical analysis showed a significantly higher percentage of responders to HCP1 in Mahajanga's samples (corrected $p<0.001$). Among positive samples, significantly higher reactivity to CPS, AhpC, and LPSB was also found, suggesting recent exposure and active circulation of *B. pseudomallei* (corrected $p<0.05$). Positive reactivity to HCP1 was observed in 46 samples from Mahajanga (4.5%, n=1009) compared to 2-9 samples from other regions (0.2-0.8%, n=1032-1553).

These findings suggest that Mahajanga is a potential hotspot for melioidosis, and that *B. pseudomallei* may be endemic. Further investigations, including *B. pseudomallei* detection in soil, are ongoing. Given the under-reported nature of melioidosis globally, these results emphasize the importance of increasing surveillance and diagnostic efforts to better understand the disease's impact in Madagascar and similar endemic areas.

Genomic Insights into *Burkholderia pseudomallei* Isolates from Central India

Singh, Jitendra[1]; Gupta, Ayush[2]; Malhotra, G Anvita[2]; Dhadeech, Malti[1]; Vishwakarma, Satyaprakash[1];

[1]Dept of Translational Medicine; [2]Dept. of Microbiology, All India Institute of Medical Sciences Bhopal, Madhya Pradesh, India-462020

Background: Melioidosis, a severe infectious disease affecting humans and animals, is caused by the bacterium *Burkholderia pseudomallei*. While primarily endemic to Southeast Asia and Northern Australia, its presence in other regions necessitates a comprehensive understanding of its genetic makeup and virulence. This study provides a pioneering genomic analysis of *B. pseudomallei* isolates from Central India, a region where the disease burden is increasingly recognized.

Methods: Six clinical isolates of *B. pseudomallei*, collected between 2020 and 2022 from Central India, underwent whole-genome sequencing using the Ion Torrent platform. Subsequent analysis focused on identifying antibiotic resistance genes, characterizing virulence factors, and detecting mutations. Single nucleotide polymorphism analysis provided insights into the genetic diversity and epidemiological relationships among the isolates.

Results: This study represents the first whole-genome sequencing effort for *B. pseudomallei* in India. Notably, all isolates exhibited susceptibility to the primary antibiotics used in melioidosis treatment. Phylogenetic analysis unveiled a high degree of genetic diversity among the strains, underscoring the dynamic nature of this pathogen. The study identified a diverse array of virulence factors, including those associated with pneumonia, liver infections, abscess formation, and septicemia. This diversity in virulence factors likely contributes to the observed regional differences in melioidosis clinical presentations, highlighting the importance of tailoring treatment strategies. Importantly, despite the genetic variability observed, all strains remained susceptible to standard treatment regimens, suggesting that antibiotic resistance in *B. pseudomallei* is not yet a widespread concern in Central India.

Conclusion: This study provides crucial insights into the genomic landscape of *B. pseudomallei* in Central India. The observed genetic diversity, coupled with the presence of a wide range of virulence factors, underscores the need for continuous surveillance to monitor the evolution of this pathogen and to adapt treatment strategies accordingly. The findings contribute significantly to our understanding of melioidosis and emphasize the importance of genomic surveillance in combating this serious infectious disease.

Keywords: *Burkholderia pseudomallei,* genetic diversity, melioidosis, antibiotic resistance, virulence factors.

Exploring the spatial population structure of *Burkholderia pseudomallei* using PubMLST data

Ward, Linda M; Kaestli, Mirjam; Mayo, Mark; Meumann, Ella M; Jolley, Keith A; Dance, David A B; Currie, Bart J; Webb, Jessica R

Global and Tropical Health Division, Menzies School of Health Research, Charles Darwin university, Darwin, Northern Territory, Australia

The global footprint of *Burkholderia pseudomallei*, the causative agent of melioidosis, has expanded to new regions including those outside of the tropics. Its geographical expansion and associated mortality have led to a great amount of interest in attempting to understand the genetic background and its epidemiology in each region. MLST is a categorical typing system based on the sequences of seven *B. pseudomallei* housekeeping genes (alleles). *B. pseudomallei* molecular typing data in PubMLST (http://pubmlst.org, operative since 2016) is widely used to classify isolates into sequence types (STs) for epidemiologic investigation. We provide a summary of the global distribution of *B. pseudomallei* STs using data from PubMLST.

As of August 2024, the PubMLST *B. pseudomallei* database contained more than 1,700 STs, and almost 6,500 isolates with linked province from 51 countries spanning more than 100 years.

A Canonical Analysis of Principal (CAP) coordinates analysis of the *B. pseudomallei* allelic profiles correctly classified 94% of (862) STs from samples collected in Oceania and 87% of (805) STs from samples collected in Asia. Eighty-nine percent of (31) STs from samples collected in Africa, 91% of (33) STs from samples collected in Europe, and 62% of (57) STs from samples collected in the Americas were classified as being from Asia. Twenty-three STs were shared between Oceania and Asia.

We demonstrate that the broad-scale geographical origin of *B. pseudomallei* can be predicted based on the allelic profile. This can enable differentiation of recent importation from unmasking of previously unrecognised endemicity.

Use of Live-attenuated and Subunit Vaccine Candidates to Evaluate Homologous and Heterologous Immunization Strategies to Combat Melioidosis

Sergei S. Biryukov[1]*, Christopher K. Cote[1]*, Christopher P. Klimko[1], Jennifer L. Dankmeyer[1], Nathaniel O. Rill[1], Melissa Hunter[1], Michael L. Davies[1], Zander M. Hedrick[1], Jennifer L. Shoe[1], Lindsey K. Schmidt[2], Caitlyn E. Orne[2], Ju Qiu[3], Mary N. Burtnick[2,4], Paul J. Brett[2,4], Susan L. Welkos[1], and David DeShazer[1]

[1]United States Army Medical Research Institute of Infectious Diseases (USAMRIID), Bacteriology Division, Fort Detrick, Frederick, MD 21702, USA; [2]Department of Microbiology and Immunology, University of Nevada, Reno School of Medicine, Reno, NV USA; [3]United States Army Medical Research Institute of Infectious Diseases (USAMRIID), Biostatistics Division, Fort Detrick, Frederick, MD 21702, USA; [4]Department of Microbiology and Immunology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

*Contributed equally

Melioidosis is a major cause of disease and mortality in tropical regions. The etiologic agent, *Burkholderia pseudomallei*, is being increasingly isolated from an expanded range of environmental and clinical sources in locations including the United States. The disease can have multi-faceted clinical presentations and requires a complex and protracted treatment regimen which is complicated by resistance of this microbe to numerous antibiotics. Thus, prophylactic countermeasures are needed, however, a vaccine has yet to be licensed for human use. In the present study, we evaluated homologous and/or heterologous combinations of live-attenuated and subunit vaccine candidates in a murine aerosol model of melioidosis to determine the effects of vaccine composition and delivery schemes on protection. Immune responses and bacterial clearance were also assessed. The vaccine candidates included a live-attenuated strain (*B. thailandensis* E555 Δ*ilvI*) or defined subunit antigens consisting of *B. thailandensis* E555 6-deoxyheptan capsular polysaccharide (CPS) covalently linked to recombinant CRM197 diphtheria toxin mutant to produce CPS-CRM197, recombinant hemolysin co-regulated protein 1 (Hcp1), and type A O-polysaccharide (OPS) conjugated to Hcp1 (OPS-Hcp1) or to CRM197 (OPS-CRM197). Both strategies resulted in significant levels of protection against lethal inhalational challenges of *B. pseudomallei*. Our findings suggest that a heterologous vaccination strategy employing CPS-CRM197 plus Hcp1 combined with *B. thailandensis* E555 Δ*ilvI* may be a promising approach for combatting disease caused by this important bacterial pathogen.

Disclaimers

The opinions, interpretations, conclusions, and recommendations presented are those of the author and are not necessarily endorsed by the U.S. Army or Department of Defense.

Contractor – this does not constitute an endorsement by the US Government of this or any other contractor.

Characterization of the target antigen TcdB for development of countermeasures against glanders and melioidosis

Sarah Hosking[1], Nichole Orr-Burks[2], Jeff Hogan[1,2], Eric Lafontaine[1]

[1]Department of Infectious Diseases, [2]Department of Biomedical Sciences, University of Georgia, College of Veterinary Medicine, Athens, GA

Burkholderia mallei (*Bm*), the causative agent of glanders, shares significant genomic similarity to *Burkholderia pseudomallei* (*Bp*), the causative agent of melioidosis, both of which are known to cause severe, potentially lethal disease in humans and animals. *Bp* and *Bm* lack a vaccine and are difficult to treat due to high levels of antibiotic resistance, necessitating the development of novel therapeutics and prophylactics. TcdB, a Tc toxin complex subunit expressed during *in vivo* glanders infection, is a putative virulence factor shared by *Bp* and *Bm* and a potentially attractive target for countermeasure development. A strain of *Bm* that lacks functional expression of TcdB, *Bm tcdB*KO, displays marked attenuation in an intracellular model of infection, evidenced by decreased syncytia formation and over a 60% reduction in plaque area. A mouse model of aerosol infection was used to ascertain TcdB's importance during *in vivo Bm* infection, in which BALB/c mice were intratracheally infected with escalating doses of WT *Bm* or *Bm tcdB*KO using a Microsprayer device and observed for two weeks post-infection or until humane endpoint was reached. Compared to WT *Bm*, *Bm tcdB*KO required 131-fold more CFUs to reach the median lethal dose. Moreover, mice infected with *Bm tcdB*KO displayed alleviated clinical symptoms and diminished bacterial burden in their lungs and spleens compared to WT *Bm*-infected mice. These data substantiate TcdB's classification as a *Burkholderia* virulence factor, and the important role that TcdB plays in *Bm* pathogenesis makes it an attractive target for *Bp* and *Bm* countermeasure development.

Immunization with *Burkholderia pseudomallei* Antigen Cocktail Protected Mice against Aerosol Challenge

Sim, Siew Hoon; Wang, Dongling; Lim, Jie Hui; Tan, Gek Yen Gladys

Biological Defence Programme, DSO National Laboratories, Singapore

Significant progress in our understanding of *Burkholderia pseudomallei* (Bp) pathogenesis and host immunity has led to the development of promising vaccine candidates against melioidosis over the last decade. Various strategies, including using killed whole cell, live attenuated, glycoconjugate, subunit and outer membrane vesicles, have been investigated though none of the vaccine candidates has progressed to human trial to date.

In this study, we evaluated the protective efficacy of an in-house formulation of Bp antigen cocktail prepared from strain K96243 (BpAg-K9) against Bp aerosol challenge. A three-dose regimen of 5µg of BpAg-K9 was administered subcutaneously to C57BL/6 mice. The mice were then challenged with aerosolized K96243 and observed for survival over a period of 32 days.

The vaccinated mice demonstrated an enhanced survival rate of 80% in comparison to 20% survival rate in non-vaccinated mice. Additionally, 100% of surviving mice exhibited complete bacterial clearance from the blood. Further immunological investigations revealed that BpAg-K9 vaccine elicited significantly higher IgG1 and IgG2c antibodies, and enhanced Th1 cell-mediated responses as evidenced by a significant increase in the percentage of IFN-γ secreting CD4-T cells and CD8-T cells compared to non-vaccinated mice. The proportion of effector memory T cell ($CD4^+CD44^+CD62L^{low}$ and $CD8^+CD44^+CD62L^{low}$) was also significantly increased, indicating the development of memory cells in response to the vaccination. Taken collectively, these results indicated that BpAg-K9 conferred protective immunity based on the activation of both humoral and cell-mediated immune responses, suggesting that it could be considered as a potential vaccine candidate for melioidosis.

Antibody production against *Burkholderia pseudomallei* antigenic proteins

Yi, Jinhee[1*]; Hansen, Holly[1]; Henry, Shantel[1]; Ndiaye, Mame Diarra Bousso[1]; Stone, Nathan E.[1]; Birdsell, Dawn; Roe, Chandler C.[1]; Wagner, David [1,2] Keim, Paul[1,2], and Settles, Erik[1,2]

[1]Pathogen and Microbiome Institute, Northern Arizona University, Flagstaff, Arizona, USA; [2]Biological Sciences, Northern Arizona University, Flagstaff, Arizona, USA

Antigenic proteins of Bp outer membrane vesicles have been identified as potential immunogens producing a humoral immune response that correlates to protection against the disease. Produced mono- and poly-clonal antibodies can serve diverse medical roles. Several proteins have been identified as potential correlates of protection, including Outer Membrane protein A, Flagellar protein, and Lipoprotein. These proteins were utilized to generate monoclonal antibodies (mAb) for vaccine evaluation and future protection studies.

Purified antigenic proteins were administered to BALB/c mice, and polyclonal antibodies (pAbs) were collected. Splenocytes collected from all the immunized mice were utilized to produce monoclonal antibodies (mAbs). Linear epitopes were also identified using a peptide-DNA tagged library. Using these antigens or epitopes, we performed Barcode-Enabled Antigen Mapping or antibody capture using nanovials. These techniques facilitated the selection/ collection of cells that generate mAb that bind to the cognate targets through B-cell receptors or secreted antibodies, facilitating single-cell sequencing to obtain antibody sequences and expression of recombinant antibodies.

An ELISA assay showed that the pAb reactivity increased over time after multiple boost immunizations. The pAbs from the final blood-draw showed maximized IgG reactivity against the target antigens. For recombinant mAbs validation, we used ELISA or western blotting. The performance analysis of mAbs demonstrated that most antibodies showed noticeable reactivity against cognate antigenic proteins. From this approach to antibody production, we could produce high-affinity antibodies that can be used in vaccine evaluation and confirm the potential correlate of antibody protection generated by the OMV vaccine.

Identifying markers of mortality by integrative analysis of acute melioidosis patients from Thailand

Priyanka Abraham, Barbara Kronsteiner-Dobramysl, Jennifer Hill, Mohammad Ali, Clement Twumasi, Martha Zewdie, Parinya Chamnan, Direk Limmathurotsakul, Nicholas P.J. Day, Paul Klenerman, Susanna Dunachie

Peter Medawar Building for Pathogen Research, Nuffield Department of Clinical Medicine, University of Oxford , Oxford, United Kingdom.

With hospitalised case fatality rate at 43% in Northeast Thailand, defining markers of disease severity and mortality in melioidosis to differentiate those who will go on to survive an acute melioidosis infection could steer early treatment interventions.

We have collected extensive clinical and immunological data including antibody, T cell and cytokine responses from a cohort of Thai adults with acute culture-confirmed melioidosis (n=81) and healthy adults from the same region (n=20). In this cohort, 57 (70%) patients with acute melioidosis had diabetes mellitus (DM) of whom 19 (33%) did not survive infection.

To identify immunological predictors of mortality, an unsupervised approach of visualisation and analysis using principal component analysis (PCA) was performed. Key molecules which accounted for 46.8% of variance in infected individuals were IL-8, IL-15, MCP-1, TNF, IL-6, IP10, CRP, IL-1RA, MIP-1a and VCAM-1. Other immunological parameters included in the analysis did not contribute as highly as the cytokines in the variance seen. Separation of individuals by mortality status showed less variability among survivors compared to fatal cases. High variability amongst those who died could be due to inter-individual differences in immunological responses and comorbidities including DM. When paired comparison was performed, all molecules except MCP-1 showed significant upregulation in died compared to survived ($p<0.05$). Next, we will perform cluster analysis to further differentiate survivors and died by incorporating clinical data to phenotype responses using the machine learning platform, Pandora. This will advance our understanding of immune correlates of protection (CoP) in melioidosis and immune features associated with DM.

Active Melioidosis Surveillance among Hospitalized Patients with Diabetes Mellitus in Bangladesh, June 2021-March 2024

Sukanta Chowdhury[1], Lovely Barai[2], Muhammad Abdur Rahim[2], Mindy Elrod[3], Maria Negron[3], Zach Weiner[3], Samira Rahat Afroze[2], Caroline Schrodt[3], Farhana Afroz[2], Julia K. Petras[3], Muhammad Belal Hossain[1], Ayesha Jamal Ananna[1], Mahmudur Rahman[1], Mohammed Ziaur Rahman[1], Jay Gee[3], William A. Bower[3]

[1]International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b); [2]Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM) General Hospital, Dhaka, Bangladesh; [3]Centers for Disease Control and Prevention, Atlanta, Georgia, USA

Melioidosis is an infectious disease predominantly found in tropical regions, caused by soil-borne bacterium *Burkholderia pseudomallei*. Melioidosis remains a neglected disease in Bangladesh. From 1961-2021, Bangladesh has documented around 85 cases. The incidence of melioidosis is believed to be much higher. To determine a more accurate incidence of melioidosis in Bangladesh, we initiated surveillance in June 2021 to detect *Burkholderia pseudomallei* infection in hospitalized patients at BIRDEM General Hospital. BIRDEM General Hospital is the largest diabetes hospital in Bangladesh which has 715 in-patient beds and more than 3500 out-patient visits daily. Adult patients with diabetes mellitus having clinical suspicions for melioidosis as per case definition were enrolled. As of 20 March 2024, a total of 693 were enrolled; 53% (n= 365) were male and the mean age of all patients was 58 years (range: 18-105). Of the 693 patients, 28 (4%) were culture confirmed for *Burkholderia pseudomallei*, 7 (25%) of whom died. The mean age of cases was 54 years (range: 25-70); male was predominant (82%), most patients (96%) had diabetes; 23 (82%) had fever; 7 (25%) had sepsis syndrome; 13 (46%) had skin abscess; 10 (36%) had pneumonia; 4 (14%) had organ abscess; 17 (61%) had urinary tract infection (UTI) and 13 (46%) had chronic Kidney disease (CKD). Most of the cases (n=22, 79%) were detected during June-November; 18 (64%) cases lived in rural areas and all cases originated from 17 districts. Patients with melioidosis were more likely to have soil exposure within the prior 30 days compared to those that did not (OR 2.6, 95% CI: 1.21-5.47). *Burkholderia pseudomallei* showed highest sensitivity to meropenem (100%), amoxicillin-clavulanate (100%), ceftazidime (100%), piperacillin (100%) followed by tetracycline (73%), cotrimoxazole (57%), and ciprofloxacin (35%). This hospital-based active surveillance provides evidence that the burden of melioidosis is higher in Bangladesh than previously documented. Active surveillance should be expanded with diagnostic facilities to understand the true country-wide melioidosis burden.

Effect of *Burkholderia pseudomallei* and *Escherichia coli* lipopolysaccharides in nitrite generation and expression of iNOS and TNF-α genes in RAW 264.7 macrophages

Gunawardane, Induwari[1]; Anuradha, Kaushalya[1]; Premawansa, Sunil[2]; Corea, Enoka[3]; Fernando, Narmada[1]

[1]Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo, Sri Lanka, [2]Department of Zoology and Environment Sciences, University of Colombo, Sri Lanka, [3]Department of Medical Microbiology and Immunology, University of Colombo, Sri Lanka

Melioidosis is a tropical infection endemic to Sri Lanka, caused by a Gram-negative bacterium, *Burkholderia pseudomallei*. Bacterial lipopolysaccharides (LPS) play a critical role in triggering immune cells and eliciting innate immune responses including the production of pro-inflammatory products. The current study compared the cellular production of nitrite ($NO2^-$; as RNS) and the molecular expression of inducible nitric oxide synthase (iNOS) and tumor necrosis factor – alpha (TNF-α) genes induced by the LPS of *B. pseudomallei* (NCTC 13177) with those induced by commercially available LPS of *Escherichia coli* 0111: B4 in cultured RAW 264.7 mouse macrophage cells. RAW 264.7 cells were stimulated with a concentration series (1-1000 ng/mL) of LPS from *B. pseudomallei* and *E. coli*. $NO2^-$ levels were determined by the Griess assay. RAW 264.7 cells stimulated with *B. pseudomallei* LPS generated $NO2^-$ levels in a range of 2.343 – 12.007 µM, whereas cells stimulated with *E. coli* LPS resulted in levels of 13.624 – 24.781 µM ($p < 0.05$). iNOS and TNF-α gene expression was determined by reverse transcriptase PCR in cellular RNA extracts of RAW 264.7 cells, stimulated with 1000 ng/mL of each LPS. *E. coli* LPS stimulated RAW 264.7 macrophages demonstrated higher iNOS/GAPDH and TNF-α /GAPDH relative gene expression (1.468 ± 0.053, 1.280 ± 0.062) compared to *B. pseudomallei* (0.574 ± 0.008, 0.501 ± 0.072). In conclusion, the optimum inducible concentration for each bacterial LPS, based on nitrite levels, was verified as 1000 ng/mL. *E. coli* 0111: B4 LPS resulted in higher levels of nitrite production and iNOS and TNF-α gene expression than *B. pseudomallei* LPS in stimulated RAW 264.7 macrophages deducing that, for RAW 264.7 macrophages, *E. coli* 0111: B4 LPS act as a better stimulant compared to *B. pseudomallei* LPS.

The Impact of Type 2 Diabetes on Unconventional T Cell Response in Acute Melioidosis

Paramita, Isanawidya H.[1,2,3]; Limmathurotsakul, Direk[2,3]; Chewapreecha, Claire [2,3]; Chamnan, Parinya[4]; Day, Nicholas P.J.[2,3]; Hamers, Raph L.[3,5]; Dunachie, Susanna J.[1,2,3,6]; Kronsteiner, Barbara[1,2,3].

[1]Peter Medawar Building for Pathogen Research, Nuffield Department of Medicine, University of Oxford, Oxford, UK; [2]Mahidol Oxford Tropical Medicine Research Unit, Mahidol University, Bangkok, Thailand; [3]NDM Centre for Global Health Research, Nuffield Department of Medicine, University of Oxford, Oxford, UK; [4]Cardiometabolic Research Group, Department of Social Medicine, Sanpasitthiprasong Hospital, Ubonratchathani, Thailand; [5]Oxford University Clinical Research Unit Indonesia, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia; [6]NIHR Oxford Biomedical Research Centre, Oxford University Hospitals NHS Foundation Trust, Oxford, UK

Individuals with type 2 diabetes (DM) are at a 12-fold increased risk of developing melioidosis. While T-cell dysfunction in DM is suspected to contribute to heightened susceptibility, most research has focused on conventional T cells. The role of unconventional T cell subsets - such as γδ T cells, MAIT cells, and invariant NK T cells - remains poorly understood, despite their importance in early infection defence. This study aimed to investigate the impact of DM on the response of unconventional T cell subsets during acute melioidosis. Peripheral blood mononuclear cells (PBMCs) were isolated from acute melioidosis patients (n=48) and endemic controls (n=49) with and without DM at Sunpasitthiprasong Hospital, Ubon Ratchathani, Thailand. A comprehensive 34-color panel was employed for *ex vivo* phenotyping of unconventional T cells, assessing activation, exhaustion, metabolism, senescence, differentiation, and functional markers. Current findings indicate a reduced frequency of MAIT cells (p=0.02) in acute melioidosis patients with DM (n=26, IQR=0.43) compared to those without DM (n=22, IQR=1.19). Additionally, a significant decrease in Vγ9Vδ2 T cell frequency (p=0.02) was observed in DM patients (n=24, IQR=1.45) compared to non-DM controls (n=25, IQR=2.44). Preliminary data on unsupervised clustering revealed an increased proportion of terminal effector memory Vγ9Vδ2 T cells (p=0.02) in acute melioidosis patients with DM (n=6, IQR=0.22) versus those without DM (n=5, IQR=0.09). Our findings highlight the influence of DM on distinct unconventional T cell signatures. These insights could inform the development of targeted vaccine strategies for this high-risk population.

Association of diabetes mellitus with the disease entity and clinical parameters in culture positive melioidosis

Piyasiri, D.L. Bhagya; Jayasekera, P. Krishantha; Corea, Enoka

National Hospital Galle, Sri Lanka

Melioidosis is a multi-system infection caused by *Burkholderia pseudomallei*, living in soil which is transmitted to humans through inoculation, inhalation or ingestion. According to numerous studies, diabetes mellitus (DM) is the major risk factor, not only predisposing to melioidosis but also altering the immune response to the infection.

Anonymised data of 94 culture positive melioidosis patients from December 2014 to July 2024 were accessed to analyse disease entity, risk factors and laboratory investigations on admission.

Among 94 patients, 66 (70%) were diabetics. Among females 100% (11) of >60yrs and 64% (7) of 40-60yrs, and 92% (33) males of 40-60yrs were diabetics. There were patients with abscesses including deep seated ones (40), pneumonia (24), septic arthritis (8),

pyelonephritis (7), and others including endocarditis (2). Mortality rate was 20/94 (21%) of which 14 (70%) were DM.

There was a significant association between abscesses/pus formation and the presence of DM (p=0.0067). Melioidosis antibody titre was tested in 75 (80%) and there was a significant association of high titres >1:160 with the presence of DM (p=0.01). Associations of ESR >100mm/1st hr and high platelet counts (>400000/µL) with DM were statistically significant (p=0.0001 and p=0.0272 respectively). Association of white cell count (WCC) >11000/µL with DM was not statistically significant (p=0.7914).

Conclusions

The prevalence of diabetes in this melioidosis cohort was 70%. Association between abscess formation and diabetes was statistically significant. The presence of DM is associated with higher antibody titres, high ESR and high platelet count which may indicate more chronic or extensive disease.

Unlocking cryptic expression of secondary metabolites and surface-associated polysaccharides in the great adapter, *Burkholderia pseudomallei*.

Sam Golon, Ian McMillan, Amr Ramadan, Grace Borlee, Brad Borlee

Department of Microbiology, Immunology & Pathology, Colorado State University, United States of America

Burkholderia pseudomallei (*Bp*) is the causative agent of melioidosis, a severe infectious disease with widespread distribution and high mortality rates. *Bp* is resistant to many antimicrobials and has the ability to persist in the environment. *Bp* is able to efficiently transit from environmental reservoirs to cause infection in a host, but little is known about the sensory adaptation response required to achieve this transition. *Bp* also produces a diverse array of surface-associated polysaccharides, which contribute to its adaptability. Additionally, it produces numerous secondary metabolites. Together, these molecules likely contribute to the ability of *Bp* to exist efficiently in diverse environments and to adapt readily to the host upon infection. Our aim is to understand how environmental cues can induce physiological changes, enabling dramatic transitions in lifestyle. To better understand the regulation of these molecules we used a variety of approaches that rely on disrupting signaling systems in *Bp* and concurrently altering the environmental conditions. Targeted disruption of c-di-GMP messaging reveals phenotypic impacts on biofilm formation, colony morphology, motility, and secondary metabolite expression in response to environmental cues. The ability to produce numerous surface-associated polysaccharides and secondary metabolites in *Bp* is context-dependent. Further probing the regulatory mechanisms underlying these complex circuits, we have also evaluated the role of two-component regulatory systems that contribute to biofilm formation and infection processes. Our approach has the potential to find the keys to understand the cryptic expression of components that contribute to environmental transition dynamics that lead to infection.

Identification and Characterization of Essential Genes in *Burkholderia pseudomallei* using CRISPRi System for Novel Drug Target Discovery

Ajimathorn, Yada;[1] Chirakul, Sinisa[2]

[1]Interdisciplinary Program of Medical Microbiology, Graduate school, Chulalongkorn University, Bangkok, Thailand; [2]Division of Bacteriology, Department of Microbiology, Faculty of Medicine, Chulalongkorn University, King Chulalongkorn Memorial Hospital, Thai Red Cross Society, Bangkok, Thailand.

Melioidosis, caused by *Burkholderia pseudomallei*, is a deadly disease with limited treatment options, highlighting the urgent need for novel antibiotics. Essential genes in *B. pseudomallei*, crucial for bacterial survival, offer promising drug targets for developing novel antibiotics against this life-threatening infection. However, genetic tools for genetic engineering are limited in this organism, and not all genes can be deleted, especially essential genes. This study employs the clustered regularly interspaced short palindromic repeat CRISPR interference (CRISPRi) system to identify and characterize essential genes in *B. pseudomallei*. To confirm the CRISPRi system's suitability for downregulating essential genes in *B. pseudomallei*, we targeted the known essential gene *ftsZ*, which encodes a cell division protein. The result showed that the CRISPRi system effectively silenced *ftsZ* expression, leading to growth inhibition in *B. pseudomallei*, thus confirming its potential for targeting other essential genes. This system was then used in the identification and characterization of candidate essential genes including BP1026B_I2635 (*lspA*) and BP1026B_I2524 (*hemC*). The decrease in mRNA level and the growth defect were observed in *B. pseudomallei* strains harboring sgRNA g4, and g1 targeting BP1026B_I2635 and BP1026B_I2524 genes, respectively. In conclusion, this study demonstrates the successful utilization of the CRISPRi system for the identification and characterization of essential genes in *B. pseudomallei*. By silencing the expression of BP1026B_I2635 and BP1026B_I2524, we confirmed their essentiality and validated the CRISPRi system's efficacy in this pathogen. These findings pave the way for further exploration of essential genes as potential novel drug targets, contributing to the development of alternative therapeutic strategies against melioidosis.

Evaluation of the bacterial virulence and host response to neurological infection in C57BL/6 mice exposed to aerosolized *Burkholderia pseudomallei* ATS2021

Christopher K. Cote[1*], Kevin D. Mlynek[1], Christopher P. Klimko[1], Sergei S. Biryukov[1], Sherry Mou[1], Melissa Hunter[1], Nathaniel O. Rill[1], Jennifer L. Dankmeyer[1], Jeremy A. Miller[1], Yuli Talyansky[1], Michael L. Davies[1], Keersten M. Ricks[2], Tamara L. Clements[2], Christina E. Douglas[2], Suma Ravulapalli[2], Taalin S. Hoj[2], Ju Qiu[3], Nancy A. Twenhafel[4], Joel A. Bozue[1], Christopher P. Stefan[2], David DeShazer[1], and Charles J. Shoemaker[2]

[1]Bacteriology Division, United States Army Medical Research Institute of Infectious Diseases, 1425 Porter Street, Fort Detrick, Frederick, MD 21702; [2]Diagnostics Systems Division, United States Army Medical Research Institute of Infectious Diseases, 1425 Porter Street, Fort Detrick, Frederick, MD 21702; [3]Biostatsistics Division, United States Army Medical Research Institute of Infectious Diseases, 1425 Porter Street, Fort Detrick, Frederick, MD 21702; [4]Pathology Division, United States Army Medical Research Institute of Infectious Diseases, 1425 Porter Street, Fort Detrick, Frederick, MD 21702

Melioidosis is an emerging infectious disease in the U.S. A subset of melioidosis patients will develop neurological melioidosis. In 2021, four cases of melioidosis associated with an aromatherapy spray imported from India were reported in the USA. Two of the cases involved children who developed neurological melioidosis. We assessed aspects of bacterial pathogenesis of isolate ATS2021 in the C57BL/6 mouse model of inhalational melioidosis. Histopathological analyses indicated that the bacteria were quickly associated with the nasal cavity and were then identified in the olfactory bulb of the brain within three days (depending upon the dose inhaled). We applied a transcriptomic approach targeted against a range of neuroinflammatory genes to brain homogenates from infected mice. We observed a pronounced inflammatory response across the high bacterial dose groups, particularly up to five days post-challenge. Some of these genes included LCN2, GBP2, and CXCL10. Of particular interest was the consistent elevation of LCN2, which is an acute-phase host response element that is secreted by astrocytes and is associated with neuronal death. We utilized a cell profiling module to identify changes in neuronal cells based on relative abundance of marker genes associated with oligodendrocytes. We observed significant drops in oligodendrocyte signatures. Twenty-seven genes constitute the oligodendrocyte profile, and 23 had significant expression differences for at least one time-point. We observed pronounced downregulation of many of these genes, as early as day one. We must understand these new clinical isolates in context of inhalational and neurological melioidosis to predict the hazards associated with this pathogen.

The opinions, interpretations, conclusions, and recommendations presented are those of the authors and are not necessarily endorsed by the U.S. Army or the Defense Health Agency.

Research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the *Guide for the Care and Use of Laboratory Animals*, National Research Council, 2011. The facility where this research was conducted is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

Funding: US Defense Threat Reduction Agency

Characterising novel bacterial factors affecting systemic dissemination of *Burkholderia pseudomallei*

Doyle, Rose; Stevens, Joanne; Moule, Madeleine G.

Institute of Infection and Immunology Research, University of Edinburgh, Edinburgh, United Kingdom

Melioidosis is known for its wide range of clinical presentations, from localised abscesses to fatal systemic infections. The most severe manifestations are associated with dissemination of *Burkholderia pseudomallei* beyond the initial site of infection. The mechanisms involved in this process are poorly understood, but roles for macrophages and dendritic cells in facilitating systemic spread have been proposed. We hypothesise that by identifying bacterial factors involved in establishing systemic infections and characterising their roles in dissemination, we can shed insight into this aspect of *B. pseudomallei* pathogenesis.

We have re-analysed an existing dataset from a large-scale genetic screen to identify genes involved in extrapulmonary dissemination of *B. pseudomallei*. Deletion mutants of candidate genes have been constructed in the model organism *Burkholderia thailandensis,* and used for *in vitro* characterisation. We hypothesise that these genes are involved in the first step in extrapulmonary dissemination – crossing the epithelial barrier of the lung – possibly by contributing to the survival of *B. pseudomallei* within migrating macrophages. We have developed an air-liquid interface (ALI) co-culture model which mimics the pulmonary epithelium to investigate this hypothesis *in vitro*.

Elucidating the role of a putative *para*-aminobenzoic acid synthesis enzyme in *Burkholderia pseudomallei*

Johnson, Ashley; Kahler, Charlene; Debowski, Aleksandra; Ireland, Philip; Stubbs, Keith; and Sarkar-Tyson, Mitali.

Marshall Centre for Infectious Disease Research and Training, The University of Western Australia, Perth, Australia

Folate metabolism has been a long-standing target of successful antimicrobials. Humans are unable to synthesise folate, making the bacterial folate biosynthesis enzymes prime targets for antibiotics. The folate molecule comprises three linked moieties: a pterin ring, *para*aminobenzoic acid (PABA), and glutamate. While the synthesis of these moieties has been well-studied in many pathogens, in *B. pseudomallei*, PABA biosynthesis remains to be fully elucidated. PABA biosynthesis is well-conserved in bacteria, and involves three enzymes: PabA, PabB, and PabC. A transposon mutagenesis study by Pilatz *et al.* (2006) using environmental *Bps* isolate E8 showed the putative *pabB* gene (*bpsl2825*) to be involved in folate metabolism, and important for infection in a murine melioidosis model. In this study, through computational and bioinformatic characterisation, *bpsl2825* is shown to encode an additional C-terminal domain with strong homology to PabC. This suggests that BPSL2825 is a dual-function enzyme containing both PabB and PabC. Interestingly, PabA does not appear to be present in *Bps*. To confirm the findings of Pilatz *et al.* in a clinically relevant strain, the full-length *bpsl2825* gene was deleted from *Bps* K96243, and the mutant strain was shown to be auxotrophic for PABA. Additionally, a murine macrophage infection model validated that *bpsl2825* plays an important role in infection in this strain. This data confirms the role of *bpsl2825* in metabolism and infection in *Bps* K96243. Further enzymatic and structural characterisation will evaluate the predicted dual-function activity of BPSL2825, and its potential as a novel target for antibiotic development can be assessed.

Caspases team up to combat *Burkholderia pseudomallei* within primary human macrophages.

Lichtenegger, Sabine; Ostermann, Laura; Zauner, Andrea; Stiehler, Julia; Debnath, Supriya; Höpflinger, Johanna; Eder, Alexandra; Kulnik, Susanne; Kleinhappl, Barbara; Herzog, Mathias; Steinmetz, Ivo

Diagnostic and Research Institute of Hygiene, Microbiology and Environmental Medicine; Medical University of Graz; Graz, Austria

The pro-inflammatory cell death pyroptosis is a double-edged sword in innate immune defence. Beneficial for the host, it restricts the intracellular niche of pathogens. However, it also massively stimulates the release of pro-inflammatory cytokines, thereby contributing to harmful inflammation, for example, during sepsis. Consequently, pyroptosis is discussed as a potential host-directed therapeutic target for infectious diseases associated with hyper-inflammation including melioidosis. Macrophages initiate pyroptosis via different inflammasomes, which consist of a pathogen recognition receptor and a caspase activating the pore-forming pyroptosis executor protein Gasdermin D. This study comprehensively elucidates the pathways that trigger *Burkholderia pseudomallei*-induced pyroptosis in primary human macrophages since these had been previously undefined. We report that caspase-1 is essential for early pyroptosis induction in human macrophages. We show that this early caspase-1-associated pyroptosis depends on the known NLRC4 inflammasome inducers BsaL and BsaK and, to a lesser extent, the flagellin FliC. Distinct from the commonly used THP-1 macrophage cell line, pyroptosis is independent of the pathogen recognition receptor NLRP3 in primary macrophages. Our data also indicate a role of caspase-8 in bacterial restriction, although, in contrast to other pathogens, we did not observe an impact on *B. pseudomallei*-induced pyroptosis. In conclusion, our data suggest that different caspases team up to combat the intracellular bacterial pathogen by presumably distinct mechanisms.

Membrane engineering of outer membrane vesicles allows tunable interactions with the innate immune system

La, Thi Hai Au[1], Dahal, Prashant[1], McMillan, Ian A.[2], Borlee, Brad[3], Norris, Michael H.[1]

[1]Pathogen Analysis and Translational Health Group, School of Life Sciences, University of Hawai'i at Mānoa, Honolulu, Hawai'i, United States of America; [2]School of Life Sciences, University of Hawai'i at Mānoa, Honolulu, Hawai'i, United States of America; [3]Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, Colorado, United States of America.

Outer membrane vesicles (OMVs) have emerged as a promising vaccine platform for several infections. The outer leaflet of OMVs are composed of lipid A. Lipid A in the outer membrane is endotoxic and activates the human innate immune response through activation of TLR4. This interaction can be exploited to generate robust vaccine responses. The outer membrane of *B. pseudomallei* contains inconsistent lipid A profiles because it is variably phosphorylated and has variable, though reduced, acylation compared to *Escherichia coli*. The heterogeneous lipid A pool produced by *B. pseudomallei* can be optimized for peak performance. By engineering the genome of the attenuated *B. pseudomallei* Bp82 coupled with controlled expression of a suite of lipid A modification genes, a consistent desirable profile of lipid A can be achieved. Allelic replacement was used to knockout lipid A modification gene. Rhamnose inducible promoter-driven genes that modify lipid A by regulating deacylations, hydroxylations, dephosphorylations, and arabinosylations were stably inserted in the genome to tune modifications. Consistent panels of lipid A were prepared and structurally verified by MALDI-TOF. Their ability to induce innate immunity were measured in cell lines. Genes were knocked out and their effect on lipid A structures was verified. Engineered OMVs showed induction of the inserted genes and evidence of conistent membrane profiles. Tunable OMV lipid A are another way to define and control the OMV immune inductive functionality in vaccine formulations.

A nitric oxide sensing two-component signalling system regulates a range of infection-related phenotypes in *Burkholderia pseudomallei*

Scurlock, Matthew; Michell, Stephen; Porter, Steven

University of Exeter, United Kingdom

Melioidosis treatment failure is likely to be at least in part due to biofilm-dwelling *B. pseudomallei*. An improved understanding of how this pathogen regulates biofilm formation could therefore open up new opportunities for successfully treating melioidosis. The antimicrobial radical nitric oxide (NO) plays a key role in host immune defences against bacteria and the ability of *B. pseudomallei* to detect and defend itself against this radical is vital to establish infection. Nitric oxide sensing proteins (NosPs) have recently emerged as key regulators of biofilm formation in many bacteria species. We hypothesised that a NosP in *B. pseudomallei* would regulate biofilm formation and also considered the possibility that it may mediate NO-protective responses. We used [γ-32P]-ATP autophosphorylation assays to show that a NosP in *B. pseudomallei* controls the autophosphorylation rate of a partner histidine kinase protein in an NO-dependent manner. The histidine kinase was found to phosphorylate a response regulator protein with a HD-GYP output domain, which is associated with c-di-GMP signalling, therefore implicating NosP in modulating c-di-GMP-regulated phenotypes. Unmarked, in-frame deletion of the genes encoding either NosP or its partner histidine kinase caused drastic changes in *B. pseudomallei* biofilm formation and NO resistance, in addition to other virulence traits such as growth and swimming motility. In this respect, deletion mutants of these proteins could be attenuated for virulence, therefore making them potential live-attenuated vaccine candidates. Alternatively, NosP or its partner histidine kinase could make for an effective novel drug target for melioidosis treatment.

Investigating and characterising cell wall deficient *Burkholderia*

Stewart, Bradley; Harding, Sarah; Mukamolova, Galina

University of Leicester, United Kingdom, Leicester

I am a third year PhD student at the University of Leicester working on a project funded by the Biotechnology and Biological Sciences Research Council and the Defence Science and Technology Laboratory. My project is focused on investigating cell wall deficient bacteria, also known as L-forms. There is a growing interest in these bacteria because of their potential link to failed treatment and relapse of infections.

L-forms are physiologically distinct cells which are produced by Gram-negative and Gram-positive bacteria when treated with cell wall targeting antimicrobials. They require osmoprotection, otherwise they lyse, however they are completely resistant to all cell wall targeting antibiotics, including those used to treat melioidosis. Upon cessation of treatment, L-forms revert back to a walled state, highlighting a clinical mechanism of disease recurrence.

I have developed methods for the generation of *Burkholderia thailandensis* L-forms by treating the bacteria with supra-lethal concentrations of meropenem or D-cycloserine in osmoprotective media. Using a combination of molecular probes and fluorescence microscopy, I have demonstrated that these L-forms are viable, contain genomic material and retain an intact cell membrane. These L-forms are round and large cells with increased metabolic activity. I am currently focussed on the investigation of the conversion from rod-shaped bacteria to L-forms using ImageStream X technology. In addition, I plan to carry out transcriptomics and proteomics analyses of L-forms to investigate the molecular mechanisms of *Burkholderia* adaptation to a cell-wall deficient state.

Association of *Burkholderia pseudomallei* lipopolysaccharide genotypes and variable virulence genes with different clinical manifestations of melioidosis

Behera, Bijayini; Jena, Jayanti; Mohanty, Srujana; Mishra, Baijayantimala; Mohapatra, Prasanta R; Nayak Saurav

All India Institute of Medical Sciences [AIIMS], Bhubaneswar, Odisha.751019, India

Burkholderia pseudomallei, accounts for a plethora of outcomes ranging from dormant seroconversion, acute septicemia and to chronic, localized infections. Few studies have addressed the association of variably expressed virulence genes such as O antigen of Lipopolysaccharide (LPS), *Burkholderia* intracellular motility factor bimA, and filamentous hemagglutinin (fhaB3) in *B. pseudomallei* genome with diverse clinical manifestations and outcome. We performed PCR to determine the LPS O-antigen type, bimA type and presence of FhaB3 from one hundred consecutive culture-positive melioidosis cases (30 deep organ abscesses, 15 community acquired pneumonia, 18 bacteraemia without focus, 10 neuromelioidosis,8 bone and joint infections and 19 subcutaneous abscesses) diagnosed between 15th April 2021 to 31st Dec 2023 and determined the association of these variably present virulence genes with clinical presentation, disease severity, and mortality. LPS A (47%) was the predominant type followed by LPS B (12%), and LPS B2 was detected from a single case of septic arthritis. 47 isolates could not be assigned to any LPS genotypes suggesting greater genetic diversity of O antigen of regional *B. pseudomallei* isolates. All isolates had *bimABp* variant and ninety-eight isolates were positive for FhaB3. Presence of LPS A was associated with neuromelioidosis *(p* value 0.047). B*imABp* and fhaB3 had no significant association with any form of the disease. On multivariate regression analysis, the odds ratio of ICU admission in patients with LPS-A and no assigned LPS were 69.1 and 18.6 respectively. Additional whole genome sequencing based studies are likely to add more insights to the virulence determinant of *B. pseudomallei.*

Novel Approaches in Managing Difficult-to-treat Burkholderia pseudomallei Bone and Joint Infections: A Case Report from India Salvage therapy with Levonadifloxacin

Gupta Neha[1], Agashe Vikas[2], Soman Rajeev[3], Vidyarthi Kandarp[4], Gupta Kailash[5]

Infectious Diseases Physician, Founder- NG Swastha and Fortis Memorial Research Institute, Gurugram, [2] Department of Orthopaedics, P.D. Hinduja Hospital & Medical Research Centre, Mumbai, [3]Department of Infectious Diseases, Jupiter Hospital, Pune, [4]Department of Orthopaedics, Paras Hospital, Gurugram, [5]Department of General Surgery, Government Medical College & Teerth Nursing Home, Rewa

Bone and joint infections (BJI) caused *by Burkholderia pseudomallei* pose significant clinical challenges due to diagnostic delays and limited treatment options. Misidentification and false-negative results with novel diagnostic tools like the multiplex PCR, Joint Infection (JI) exacerbate these issues. Traditional treatments often pose intolerability and adverse effects, necessitating need for new therapeutic alternatives.

A 59-year-old gentleman presented with acute right forearm pain in November 2023. Elevated C-reactive protein levels (61.5 mg/L) led to empirical cefixime treatment for suspected typhoid fever initially. Persistent pain revealed proximal right radial osteomyelitis with elbow joint effusion on MRI. While the initial blood cultures were negative, the JI panel from the right elbow joint effusion detected *Streptococcus sp.* resulting in misdiagnosis and prompting initial treatment with ceftriaxone and linezolid. But, recurrent symptoms prompted further imaging, revealing progression of osteomyelitis with intramedullary abscess and radial head fracture. Repeat blood cultures identified *B. pseudomallei*. Surgical debridement had to be done twice for complete source control. During therapy, limited antibiotic options and drug intolerance complicated management. Severe allergies to ceftazidime, TMP/SMX, and leucopenia with meropenem, necessitated the use of oral prodrug alaevonadifloxacin for six months due to its efficacy, bone penetration, and tolerability. This is the first reported case using levonadifloxacin and oral alalevonadifloxacin *for B. pseudomallei* infection.

This case underscores the complexities in diagnosing and managing *B. pseudomallei* bone infections. Clinicians must maintain high suspicion in endemic areas, prioritize aggressive surgical intervention, and tailor antibiotic therapy to improve outcomes. The successful use of levonadifloxacin highlights its potential in melioidosis treatment.

Keywords: Burkholderia pseudomallei, osteomyelitis, melioidosis, levonadifloxacin, diagnostic challenges.

Bone and joint infections due to melioidosis; diagnostic and management strategies to optimise outcomes.

Parvati Dadwal[1, 2], Brady Bonner[3], David Fraser[4], Jeremy Loveridge[2,5], Grant Withey[6], Arvind Puri[1], Simon Smith[7], Josh Hanson[7,8 *].

[1]Department of Orthopaedic Surgery, Cairns Hospital, Cairns, Queensland, Australia; [2]James Cook University, Cairns, Queensland, Australia; [3]Department of Orthopaedic Surgery, Logan Hospital, Brisbane, Queensland, Australia; [4]Department of Orthopaedics, Prince Charles Hospital, Brisbane, Queensland, Australia; [5]Far North Queensland Hand Surgery, Cairns, Queensland, Australia; [6]Department of Radiology, Cairns Hospital, Cairns, Queensland, Australia; [7]Department of Medicine, Cairns Hospital, Cairns, Queensland Australia; [8]The Kirby Institute, University of New South Wales, Sydney, NSW, Australia

Bone and joint infections (BJI) are a recognised, but incompletely defined, manifestation of melioidosis that are associated with significant morbidity and mortality in resource-limited settings.

We identified all individuals with BJI due to melioidosis managed at Cairns Hospital, Australia between 01/01/1998 and 01/06/2023. The patients' demographics, their clinical findings and their treatment were correlated with their subsequent course.

Of 477 culture-confirmed cases of melioidosis managed at the hospital during the study period, 39 (8%) had confirmed BJI; predisposing risk factors for melioidosis were present in 37/39 (95%). In multivariable analysis only diabetes mellitus was independently associated with the presence of BJI (odds ratio (95% confidence interval): 4.04 (1.81-9.00), p=0.001). Bacteraemia was present in 31/39 (79%) and 29/39 (74%) had infection involving other organs. Of the 39 individuals with BJI, 14 (36%) had osteomyelitis, 8 (20%) had septic arthritis and 17 (44%) had both osteomyelitis and septic arthritis; in 32/39 (83%) the lower limb was involved. Surgery was performed in 30/39 (77%). Readmission after the initial hospitalisation was necessary in 11/39 (28%), 5/39 (13%) had disease recrudescence and 3/39 (8%) had relapse; 4/39 (10%) developed pathological fractures. ICU admission was necessary in 11/39 (28%) but all 11 of these patients survived. Only 1/39 (3%) died, 138 days after admission, from his significant underlying comorbidity.

The case-fatality rate from melioidosis BJI in Australia's well-resourced health system is very low. However, recrudescence, relapse and orthopaedic complications are relatively common and emphasise the importance of multidisciplinary care and thorough extended follow-up.

Comparison of the characteristics and clinical course of patients with bacteremia due to *Burkholderia pseudomallei*, *Escherichia coli* and *Staphylococcus aureus* in tropical Australia

Kelly Baker,[1] Alistair Lau, [2] Arish Soogrim, [1] Tej Shukla, [1] Jasraaj Singh, [1] Felix Torrance, [1] John Maclean, [1] Matthew Law,[3] Simon Smith, [1] and Josh Hanson.[1,3]

[1]Department of Medicine, Cairns Hospital, Cairns, Queensland Australia; [2]James Cook University, Cairns, Queensland, Australia; [3]The Kirby Institute, University of New South Wales, Sydney, NSW, Australia

The 28-day mortality rate of *Burkholderia pseudomallei* bacteraemia in Thailand has been reported to be 66%, higher than the 28-day mortality rate of bacteraemia due to *Staphylococcus aureus* (43%) and *Escherichia coli* (19%) in that country. We examined cases of bacteraemia due to *B. pseudomallei, E. coli* and *S. aureus,* admitted to Cairns Hospital in tropical Australia, to compare the characteristics and clinical course of Australian patients with blood stream infections due to these three pathogens. We also examined the contribution of age, gender, comorbidity, remote residence and First Nations Australian status to outcomes.

In total there were 164 (9.1%) episodes of bacteraemia due to *B. pseudomallei*, 650 (36.0%) due to *S. aureus* and 990 (54.9%) due to *E. coli*. There were 139/1804 (7.7%) who died within 30 days; 37/139 (27%) died within 72 hours of admission. *B. pseudomallei* had the greatest 30-day mortality (19/164 (11.6%)) of the three pathogens. In multivariate analysis, that included all five pre-specified patient characteristics, the year of presentation and the three pathogens (with *E.coli* as the reference), *B. pseudomallei* bacteraemia (HR (95%CI): 2.71 (1.59-4.61), p<0.0001), *S. aureus* bacteraemia (HR (95%CI): 2.44 (1.69-3.52), p<0.0001), severe comorbidity (HR (95%CI): 2.33 (1.53-3.54), p<0.0001) and age (HR (95%CI): 1.24 (1.10-1.40), p=0.001) were independently associated with a higher 30-day mortality, while remote residence (HR (95%CI): 0.52 (0.37-0.76), p=0.001) was associated with a lower 30-day mortality. The 30-day mortality rate of *B. pseudomallei* bacteraemia is greater than that of other pathogens and even in Australia's well-resourced health system is 11.6%.

Mycotic aneurysms due to Burkholderia pseudomallei in Australia: a case series and review of the literature.

Rhona Boyle,[1] Grant Withey,[2] Simon Smith, [1] and Josh Hanson.[1,3]

[1]Department of Medicine, Cairns Hospital, Cairns, Queensland, Australia; [2]Department of Radiology, Cairns Hospital, Cairns, Queensland, Australia; [3]The Kirby Institute, University of New South Wales, Sydney, NSW, Australia

Since 2019, *Burkholderia pseudomallei* has been the most common cause of mycotic aneurysm at Cairns Hospital in tropical Australia; a mycotic aneurysm has been diagnosed in 8/251 (3.2%) culture-confirmed cases of melioidosis. All 8 patients had at least 1 risk factor for melioidosis, all 8 had either established vascular disease (or risk factors for it) and all 8 presented during the local wet season or shortly thereafter. Although the patients were managed in a well-resourced, high-volume melioidosis centre, the diagnosis of mycotic aneurysm was frequently delayed. The patients had a turbulent course: 6/8 (75%) required ICU admission and 7/8 (88%) required surgical intervention. While all 8 patients survived to hospital discharge, 2 (25%) ultimately died from their infection, a high case-fatality rate by contemporary Australian standards. Adverse drug reactions were documented in 4/7 (57%) who commenced oral trimethoprim-sulfamethoxazole (TMP-SMX) eradication therapy; an additional 2 patients were unable to adhere to their prescribed TMP-SMX, one of whom died from relapsed melioidosis. Mycotic aneurysm is an infrequent manifestation of *B. pseudomallei* infection. It is challenging to diagnose and has a high attributable mortality. The diagnosis should be considered in patients >40 years of age who reside in, or who have travelled to, endemic areas and who present with fever and abdominal or back pain with risk factors for melioidosis and vascular disease. Early, comprehensive imaging, thorough microbiological evaluation, prompt vascular surgery review, targeted antimicrobial therapy, close longitudinal follow-up and strategies to ensure patients' retention in care are crucial to achieve optimal outcomes.

Prosthetic valve infective endocarditis due to *Burkholderia pseudomallei*: a case report and review of the literature.

Callum Pownell,[1] Benjamin E. Marsden,[1] Willis Lam,[1] Simon Smith[1] & Josh Hanson. [1,2 *]

[1]Department of Medicine, Cairns Hospital, Cairns, Queensland Australia; [2]Kirby Institute, University of New South Wales, Sydney, New South Wales, Australia

Endocarditis is a very rare complication of *Burkholderia pseudomallei* infection and prosthetic valve endocarditis rarer still. We present, to our knowledge, the first confirmed Australian case of prosthetic valve endocarditis in a patient with melioidosis. Blood cultures were persistently positive for *B. pseudomallei*, a transoesophageal echocardiogram demonstrated a mobile vegetation on his bioprosthetic aortic valve, and computed tomography and magnetic resonance imaging identified no other focus of infection. The significant rate of life-threatening relapse in patients with melioidosis necessitates extended courses of antibiotic therapy with activity against *B. pseudomallei*, an organism with intrinsic resistance to many commonly prescribed agents. Infection of prosthetic material appears to be rare in cases of melioidosis, however, if present, its removal should be strongly considered to effect cure. Fortunately, in our case, clinical response to 8 weeks of intravenous therapy and 6 months of high-dose oral co-trimoxazole obviated the requirement for valve replacement surgery. However, sadly, the patient died from complications of a stroke 4 months after cessation of his antibiotic treatment. The stroke was not felt to be directly related to his infection; however, his clinical course highlights the importance of aggressively addressing the comorbidities that predispose an individual to melioidosis and which also increase their risk of premature death.

Clinical implications of high melioidosis serology IHA titre: A 20-year retrospective study from the Top End of the Northern Territory

Ho, Cassandra; Woerle, Celeste; Freeman, Kevin; Mayo, Mark; Meumann, Ella M; Baird, Robert W; Currie, Bart J

Menzies School of Health Research, Flinders University NT Medical Program, and Royal Darwin Hospital, Darwin, NT, Australia

Melioidosis, infection with the bacterium *Burkholderia pseudomallei*, is highly endemic in the Top End of the Northern Territory of Australia. The indirect haemagglutination assay (IHA) is the most widely used serology test globally but it is not standardised between the limited number of laboratories that perform it. While concerns have been raised about the sensitivity of IHA early in melioidosis infections, the advantage of IHA over more recently developed ELISAs is that testing serial dilutions allows a titre to be recorded. While a titre of 1:40 or higher is considered positive, the specificity at these low positive titres remains uncertain. However, a high titre is considered to represent recent or past true infection with *B. pseudomallei*, rather than cross-rection with other environmental *Burkholderia* species. Also, the natural history of IHA titres over time, in both asymptomatic infection and in melioidosis has been little studied. We have assessed the clinical status and serology time courses of all 534 patients who had an IHA titre of 1:640 or higher, over a 20-year period. Of these, 324 (60.7%) were diagnosed with culture-confirmed melioidosis, with varying time courses of diagnosis of melioidosis in relation to the high serology. Of the 210 without confirmed melioidosis, 22 (10.5%) were considered highly likely to be melioidosis despite being culture negative and these were all treated as melioidosis. In the remainder, titres mostly gradually decreased over time, but the majority remained seropositive. A small number who had not been treated for melioidosis continued to have high IHA titres over years and activation from latency with a new diagnosis of melioidosis was occasionally documented. This study highlights the importance of a full clinical workup in those found to have high titre melioidosis serology as well as subsequent close clinical surveillance and yearly IHA in those not confirmed or treated as melioidosis.

From Head to Toe: Menacing Melioidosis and it's Multifocal Manifestations

McLachlan, Aidan; Gottlieb, Thomas; Cheong, Elaine; Merlino, John; Gray, Timothy; McKew, Genevieve; Beresford, Rohan; Thakkar, Heeral; Hettiarachchi, Ravin

Infectious Diseases/Microbiology Department of Concord Repatriation General Hospital, Sydney, Australia

A 69 year-old of Thai descent presented to hospital in Sydney with fevers and left foot pain precluding mobility. This occurred on a background of diabetes, dyslipidaemia, and hypertension.

He was treated as a diabetic foot infection with IV amoxicillin-clavulanate. On day 2 of admission, blood cultures yielded a bipolar staining Gram-negative bacillus which was identified by MALDI-TOF-MS (Bruker Biotyper sirius one) as Burkholderia thailandensis, in the absence of the IVD Library Extension, this was later identified as B. pseudomallei by targeted PCR at a reference laboratory.

Antibiotic therapy was changed to IV meropenem, initially 1g TDS. A repeat examination revealed a purulent right buttock abscess which also grew B. pseudomallei. Septic arthritis of the ankle was confirmed by sterile aspiration and he underwent two arthroscopic debridement surgeries. His blood cultures remained positive until day 9 of admission.

On day 9 of admission, he developed a reduced level of consciousness and upper motor neuron signs. MRI brain revealed multifocal cerebral cortical lesions and brainstem encephalitis. He was commenced on co-trimoxazole in addition to IV meropenem at 2g TDS and was admitted to the Intensive Care Unit for airway monitoring. Meropenem was changed to 8g IV continuous infusion due to subtherapeutic trough levels.

This case highlights the protean manifestations of melioidosis and the difficulty with diagnosis and treatment in non-endemic areas.

Differences in simple inflammatory markers and outcomes between pulmonary and extra-pulmonary melioidosis

Nguyen-Ho Lam[1, 2], Hoang-Thi Hong-Linh[1], Tran-Thi Thanh-Thu[3]

[1]University of Medicine and Pharmacy at Ho Chi Minh City, Vietnam; [2]Cho Ray hospital, Vietnam; [3]Children hospital No.1, Ho Chi Minh City, Vietnam

Background

Melioidosis is an infectious condition with high mortality that varies depending on the specific clinical presentation, pulmonary melioidosis (PM) including primary and secondary pneumonia versus extra-pulmonary melioidosis (ETPM). Our study aims to compare outcomes between PM and ETPM in Vietnamese patients and describe differences in simple inflammatory markers between these two groups.

Methods

A cross-sectional study was conducted at Cho Ray hospital, Southern Vietnam. All adult patients with culture-confirmed melioidosis from 06/2019 to 12/2021 was evaluated.

Results

We documented 270 melioidosis cases in which there were 158 PM cases (male/female 209/61, mean age 50 ± 13). Clinical presentation of ETPM included abscesses in liver, spleen, kidney, prostate, parotid gland, and soft tissue and joint infection. Our study showed that PM patients were the higher rates of septic shock (42.40% *vs.* 7.14%, $p < 0.001$), need for intensive respiratory or vasopressor support (39.87% *vs.* 7.14%, $p < 0.001$), and death (37.34% *vs.* 8.03%, $p < 0.001$) than those with ETPM. Although there was no significant difference about white blood cell count, neutrophil count, and neutrophil-to-lymphocyte ratio between two groups, lymphocyte count and platelet count were lower in PM group significantly ($p < 0.001$ and $p = 0.001$, respectively). The level of C-reactive protein was higher in PM group (214.63 mg/l ± 109.77 vs. 143.79 mg/l ± 92.93, $p < 0.001$).

Conclusion

PM is a severe clinical entity with strong inflammatory response that we should concern to plan the suitable strategies of intervention and prevention.

Characterizing the typical patient with melioidosis: a systematic review of demographic and clinical profiles across the globe

Paul, Prasanjit S; Savelkoel, Jelmer; Virk, Harjeet S; Krugers Dagneaux, Maarten ML; Spijker, Rene; Wiersinga, W Joost; Birnie, Emma

Amsterdam UMC location University of Amsterdam, Amsterdam, the Netherlands

Background:

Melioidosis is an often-fatal tropical infectious disease that is frequently misdiagnosed due to its diverse clinical presentations. Here, we aimed to delineate global differences in demographic and clinical characteristics of melioidosis.

Methods:

We performed a systematic review to identify human melioidosis cases between 1990 and 2022. Quantitative data of culture-confirmed cases were extracted, including demographics, risk factors, clinical manifestations, hospital admission, mortality and post-infectious sequelae.

Results:

We included 707 articles with 23,316 patients after manual deduplication. While most cases originated from Southeast Asia and Pacific (~99%), only 0.72% of cases were reported in Africa, the Americas and Eastern Mediterranean. The mean age was 41 years with a male-to-female ratio of 2:1. Diabetes mellitus was the most prevalent risk factor, present in 42% of cases. Pneumonia was present in 35% of cases, abscess formation 16% and central nervous system involvement 1%. Bacteremia occurred in 56%, followed by sepsis 14% and septic shock 5%. Overall mortality was 29%. Post-acute infection sequelae were reported in only 4% of cases.

Discussion:

Our global melioidosis registry is the largest to date, allowing for comparisons between clinical presentations, geographical regions, treatment strategies and post-infectious sequelae. Considering the frequent absence of surveillance systems in endemic countries, it is important we standardize melioidosis case reports to enhance data capture for surveillance. Data from travel-related cases can reveal both endemic and non-endemic regions associated with a risk of acquiring melioidosis. Future studies may illustrate the expansion of melioidosis to previously non-endemic areas by linking the current data to patterns in climate change.

Clinical presentation and outcome from melioidosis outbreak cases in Samarahan, Sarawak, 2023.

Authors:

Tonnii Sia[1], Hock-Hin Chua[1], Andrew Chang[1], Han-Hua Lim[1], Mong-How Ooi[2]

1Infectious diseases unit, Department of Medicine, Sarawak General Hospital;
[2]Department of Paediatric, Sarawak General Hospital.

Burkholderia pseudomellei (*Bp*) is a soil-dwelling pathogen. Human infection occurs through contact with *Bp*-contaminated water and soil, which resulted in clinical infection, melioidosis. In the study, we report an outbreak occurred from September 2022 to June 2023 in a non-melioidosis endemic area, namely Samarahan District of Sarawak, Malaysia. Samarahan District is a satellite city with rapid development over the past 20 years. There were no melioidosis cases reported from the region until September 2022. A total of 29 culture-confirmed melioidosis cases recorded in this outbreak. The median age was 36-year-old with IQR of 18.5-56.3 but the age ranged from 8-month-old to 72-year-old. 62.1% male and 37.9% had pre-existing clinical risk factors for melioidosis. Pneumonia was the main presenting symptoms (69%), followed by bacteraemia without primary foci of infection (17.2%). 31% of the cases had symptoms more than 14 days, and 48% had symptoms between 6-14 days. 65.5% presented with septic shock, 24.1% suffered from acute kidney injury, 75.9% required supplementary oxygen or ventilatory support. Only 17.2% received melioidosis active antimicrobial upon admission. 82.8% were diagnosed through blood culture, 6.9% through respiratory tract secretion and 10.3% through pus culture. Mortality rate was 20.7%. 27.5% of the cases reported recent construction projects around the housing areas or grossly contaminated water supply. 62.1% drank un-boiled water. The potential source of infection was *Bp*-contaminated water supply due to recent development, and involvement of extremely young children. The outbreak resolved with public education on drinking boiled water, communication with local authorities for water quality control.

International Multicenter Evaluation of a Second-Generation Immunochromatography Test for the Serological Diagnosis of Melioidosis

Binmaeil, Hasyanee; Bunsong Thanakon; Vongsouvath, Manivanh; Robinson, Matthew; Phommasone Koukeo; Trinh, Thanh Trung; Podin, Yuwana; Sia, Tonnii Corea, Enoka Norton, Robert; Cunningham, Sophie; Hashim, Rohaidah; Hii, Shirley Yi Fen Esa, Habib Abdul Hakim; Yek, Christina; Sam, Jamal I-Ching; Currie, Bart; Meumann, Ella; Woerle, Celeste; Brett, Paul; Burtnick, Mary; Chantratita, Narisara

Mahidol University, Bangkok, Thailand

Culture of *Burkholderia pseudomallei* is the current gold standard for diagnosis of melioidosis, but its sensitivity is low. Additionally, the serological indirect hemagglutination test has been shown to be unreliable due to its low sensitivity and specificity. A reliable and rapid test is crucial for the effective detection and management of melioidosis. To address this, we developed a second-generation immunochromatography test (ICT) to detect IgG antibodies against hemolysin co-regulated protein 1 (Hcp1) from *B. pseudomallei*. This study aims to evaluate the performance of Hcp1-ICT in an international multi-center setting. We evaluated the sensitivity and specificity of the second-generation Hcp1-ICT using stored serum samples from 1,040 individuals across Thailand, Laos, Vietnam, Malaysia, Cambodia, Sri Lanka, and Australia, with the culture serving as the gold standard. The performance of the Hcp1-ICT varied significantly across different settings. The sensitivities in Thailand, Laos, Vietnam, Malaysia, Cambodia, Sri Lanka, and Australia were 86.7%, 65.8%, 76.2%, 79.1%, 90.0%, 88.6%, and 45.8%, respectively, while the specificities were 89.3%, 98.3%, 100%, 86.7%, 100%, and 100%, respectively. Across the seven countries, the average accuracy was 82.0% (95% CI, 76.9%-89.6%). These findings suggest that while the Hcp1-ICT may be a useful diagnostic tool for melioidosis, its effectiveness may differ amongst regions. Prospective validation in diverse populations will be required to optimize its diagnostic utility.

Burkholderia colony morphotype variation: an essential but understudied phenomenon

Pauline M.L. Coulon[1]

[1]Australian Institute for Microbiology and Immunology, Faculty of Science, University of Technology Sydney, NSW, Australia

Burkholderia, particularly the *Burkholderia cepacia* complex (Bcc) and "*pseudomallei*" groups, are a significant concern due to its high antibiotic resistance and its ability to respectively, cause "*cepacia* syndrome" in immunosuppressed and cystoc fibrosis (CF) patients, melioidosis in humans with pre-disposed illness and in equines. These infections pose a considerable threat to both human and animal health, burdening the Australian healthcare system and economy. As *Burkholderia* infections are linked to the wet season and increased flooding in Eastern Australia, the rate of infections has risen, emphasizing the need for new therapeutic options.

Burkholderia's phenotypic plasticity allows it to inhabit diverse niches, including soil and hosts, facilitated by its large genomes and secondary metabolite production. This plasticity can manifest as colony morphotype variation (CMV; including phase variation), observed in pure cultures, long-term CF infections, and *B. pseudomallei* infections. CMV affects *Burkholderia*'s interactions with hosts during infection. While genomic variations, regulatory processes, and epigenetic factors like DNA methylation are known to cause CMV, the specific mechanisms and impacts of phase variation in *Burkholderia* during infection remain unknown.

This study aims to provide a comprehensive analysis of CMV in *Burkholderia*, including B. pseudomallei, by examining *B. pseudomallei* and Bcc clinical isolates. Through Omics analyses, high-throughput phenotypic assays, and infection models, we will assess the modulation of virulence factors and pathogenicity in variant-positive strains. This data will help classify variants and uncover the mechanisms of CMV during infection. The findings will aid in identifying biomarkers for investigating virulence, severity, persistence, and surveillance, and in developing targeted antibiotic therapies and vaccines that address phenotypic plasticity in *Burkholderia*.

Automated molecular detection of B. pseudomallei in contrived clinical samples

Gassiep, Ian[a,b,c]; Harris, Patrick NA[a,c]

[a]The University of Queensland, Faculty of Medicine, UQ Centre for Clinical Research, Royal Brisbane & Women's Hospital, Queensland, Australia; [b]Department of Infectious Diseases, Mater Hospital Brisbane, Queensland, Australia; [c]Pathology Queensland, Royal Brisbane & Women's Hospital, Queensland, Australia

Background: The diagnosis of melioidosis is time critical due to the organism's intrinsic antimicrobial resistance and requirement for directed therapy. Approximately 27-44% of culture-confirmed cases are not bacteraemic, therefore requiring culture diagnosis from alternate sites.

Aim: This study aimed to assess the ability of an automated molecular diagnostic instrument to detect B. pseudomallei directly from clinical samples.

Methods: Clinical samples including urine, sputum, swabs, and Ashdown broth (ASH) were spiked with known concentrations of B. pseudomallei and then processed on the Panther®.

Results: B. pseudomallei was detected in all clinical sample types tested. Contrived urine samples demonstrated the lowest limit of detection (LOD) of 1.8 x102 CFU/ml. Compared with dry swabs (LOD: 1.0 x103 CFU/ml), Amies gel agar swabs were inferior (LOD: >3.3 x104 CFU/ml). Inoculation of dry swabs into ASH for 4- and 6-hours did not improved detection. Due to the nature of the sample type the results for sputum were inconsistent. The LOD for an homogenous sample is likely to be 4.0 – 5.0 CFU/ml.

Conclusion: This study demonstrates the ability of the Panther® to detect B. pseudomallei directly from multiple clinical sample types and identifies the approximate concentration of organism required for diagnosis.

A commercial ELISA test used to assess past environmental exposure to *Burkholderia pseudomallei* reveals strong heterogeneity in animals in Thailand, an endemic country for melioidosis

Guernier-Cambert, Vanina[1,2]; Chantratita, Narisara; Thongdee, Metawee; Tuanyok, Apichai; Kamyingkird, Ketsarin; Cagape, Charlene Mae; Kaewrakmuk, Jedsada; Jhaiaun, Pairpailin; Thaipadungpanit, Janjira; Laroucau Karine

[1]Sorbonne University, IRD, INRAÉ, CNRS, UPEC, UMR iEES-Paris, 75005 Paris, France; [2]Faculty of Veterinary Technology, Kasetsart University, Bangkok, Thailand

Environmental detection of *Burkholderia pseudomallei* is a key indicator of high-risk areas for melioidosis. However, the ecological niche of this bacterium remains poorly understood, complicating efforts to identify and prioritize high-risk areas. In endemic regions such as Thailand, high seropositivity have been observed in healthy individuals, suggesting that serology may help to assess past exposure. Hence, serology in animals could serve as a sentinel surveillance method to identify areas where the bacterium is present.

The indirect hemagglutination assay (IHA), commonly used for serological diagnosis of melioidosis, has significant limitations (e.g., >26% of patients do not seroconvert with IHA). Here we used the ELISA kit "ID Screen® Glanders Double Antigen Multi-species" to test sera from horses (n=60), cattle (n=88), goats (n=102), pigs (n=38) and dogs (n=60) collected from different regions and provinces in Thailand. Our results showed heterogeneity in the distribution of seropositive samples both between and within species in different farms and geographical areas, suggesting different levels of exposure. Results from pig sera collected during a melioidosis outbreak were compared with IHA. ELISA testing of 20 human sera, including patients and healthy individuals, provided insight into the relevance of this test for detecting past exposure to *B. pseudomallei* in humans.

Further analysis is required to confirm the absence of cross-reactivity, particularly with non-pathogenic environmental *Burkholderia* spp. Our study highlights the potential of this ELISA kit as a more sensitive and accurate tool for detecting *B. pseudomallei* exposure in animals and humans, potentially improving surveillance and risk assessment in endemic regions.

Finding the *Burholderia pseudomallei* needle in the clinical laboratory haystack

Inglis, Tim JJ, McFadden, Ben, Paton, Teagan F, Adams, Lindsay, PathWest Laboratory Medicine WA, Medical School, University of Western Australia, WA Country Health Service and Health Department of WA Business Intelligence Unit.

Septicaemic melioidosis is so rare in Western Australia that frontline clinical staff and regional laboratories struggle to recognise and detect the infection soon after initial presentation. A concentration of specialist clinical and microbiology services outside the melioidosis endemic zone add to the mismatch between clinical problem and service capability. We examined annual laboratory data since the introduction of a new, statewide laboratory information system to determine the principal delays in *Burkholderia pseudomallei* positive blood cultures. Noting that the majority of blood cultures in regional centres are collected in emergency departments, and are out of hours specimens, the best return on effort is targeted educational messaging. The year-round, sporadic occurrence of septicaemic melioidosis in WA means that messaging should not be restricted to the northern wet season. Heavy reliance on centralised laboratory services in Metropolitan Perth for septicaemic melioidosis fails to recognise the urgency of early delivery of actionable interim blood culture results. Addressing this challenge requires a commitment to improve near-to point-of-care blood culture processing in regional Western Australia. Though not a replacement for early identification of *B. pseudomallei* infection, the use of machine learning algorithms to process non-microbiology laboratory data may assist initial triage of early bacteraemic melioidosis cases.

Detection of IgM by ELISA improves diagnosis of melioidosis in Malaysian children

Mohan, Anand; Rosli, Nabila Farina; Rajandran, Thilagam; Tan, Lee See; Chien, Su Lin; Abdul Halim, Siti Nur Asyiqin; Lapok, Harleyssa; Ooi, Mong How.

Bintulu Hospital, Bintulu, Sarawak, Malaysia

Background.

Although the sensitivity and specificity may be poor and predictive values are not known, serology is still widely used in the diagnostic work-up of patients with suspected melioidosis in Malaysia.

Methods.

We analysed the clinical characteristics and final diagnosis of patients who had melioidosis serology performed in Bintulu Hospital from 2017 until 2022. Serum immunoglobulin M (IgM) to *Burkholderia pseudomallei* was detected by an enzyme-linked immunosorbent assay (ELISA) performed in the national reference laboratory in Kuala Lumpur. A titre ≥ 1:320 was reported as positive.

Results.

During the 5-year period, 1455 patients were investigated for melioidosis with ELISA for *B. pseudomallei* IgM. Of these, 119 (8.2%) had culture-confirmed melioidosis, including 24 (20%) children aged < 15 years. The sensitivity of the ELISA IgM against bacterial culture was 88% (21/24) and 34% (32/95) in children and adults, respectively ($P < 0.001$). In children, specificity of the ELISA IgM in culture-confirmed melioidosis was 61%, with a positive (PPV) and negative predictive value (NPV) of 20% and 98%, respectively. When children with probable melioidosis (presence of small liver and/or spleen abscesses or negative cultures on first presentation but represented with culture-proven melioidosis) were included in the analysis, the sensitivity, specificity, PPV, and NPV was to 77%, 69%, 45% and 90%, respectively. In comparison, bacterial culture had a sensitivity, specificity, PPV, and NPV of 37%, 100%, 100%, and 82%, respectively.

Conclusions.

When used in conjunction with bacterial cultures, the ELISA for *B. pseudomallei* IgM performed in Malaysia may improve diagnosis of pediatric melioidosis.

Direct detection of *Burkholderia pseudomallei* in pus specimens of patients with suspected pyogenic melioidosis using a polymerase chain reaction

Mohanty, Srujana[1]; Behera, Bijayini[1]; Mishra, Tushar Subhadarshan[2]; Mohapatra, Prasanta Raghab[3]

[1]Department of Microbiology, [2]General Surgery and [3]Pulmonary Medicine & Critical Care
All India Institute of Medical Sciences Bhubaneswar, Odisha, India

At present, melioidosis, caused by the saprophytic gram-negative bacilli *Burkholderia pseudomallei,* remains a fatal disease with no effective vaccine or simple therapeutic regimen available for complete cure. Often, diagnosis is delayed because of a lack of essential clinical and laboratory knowledge, lack of suitable diagnostic tests, and inadequate reporting procedures. Though culture is the gold standard test, it is a highly time-consuming and challenging procedure that reflects poor outcomes. As focal pyogenic infections account for approximately 25%-40% of melioidosis cases which may later disseminate, we performed this study to assess the application of a conventional polymerase chain reaction (PCR) for direct detection of *Burkholderia pseudomallei* on pus specimens of suspected pyogenic melioidosis patients as a rapid diagnostic procedure. The study conducted over a period of 2-years from July 2022 to June 2024 in a tertiary care hospital in eastern India, included patients presenting with various pyogenic infections, such as deep organ abscesses, joint effusions, suppurative lymphadenopathy, empyema, and other localized lesions. Aspirated pus specimens or exudates were subjected to a conventional PCR targeting the Type-III secretion system gene cluster along with conventional culture techniques. Of 164 patient specimens, *B. pseudomallei* was isolated in 21 (12.8%), out of which PCR was positive in 18 specimens (sensitivity, 85.7%). All culture-negative specimens tested negative by PCR (specificity, 100%). Use of PCR in clinical laboratories for direct detection of *Burkholderia pseudomallei* has the potential to improve the timeliness of diagnosis that would enable earlier institution of appropriate treatment and saving of lives.

Role of pentraxin-3 on immune physiology in (experimental) melioidosis

Paul, Prasanjit S; Davoudian, Sadaf; Garlanda, Cecilia; Sironi, Marina; Pasqualini, Fabio; Mantovani, Alberto; Limmathurotsakul, Direk; Bottazzi, Barbara; Birnie, Emma; Wiersinga, W Joost

Amsterdam UMC location University of Amsterdam, Amsterdam, the Netherlands

Background:

Pentraxin-3 (PTX3) is an acute-phase protein that modulates protection against infectious bacterial diseases. In bacterial sepsis, PTX3 plasma levels are associated with severity, patient survival, and response to therapy. We aimed to assess the role of PTX3 in melioidosis, an important cause of pneumosepsis.

Methods:

PTX3 levels were quantified in plasma of 34 culture-confirmed melioidosis patients and 31 matched healthy controls from Sunpasitthiprasong Hospital, Ubon Ratchathani, Thailand. In additional experiments, PTX3 levels were assessed in tissue from melioidosis patients after H&E- and immuno-staining. Further, wild-type C57Bl/6j mice were intranasally inoculated with *Burkholderia pseudomallei* strain 1026b and PTX3 levels and tissue pathology were assessed in organs harvested after 24-, 48-, and 72-hours post infection.

Results:

Melioidosis patients exhibited elevated PTX3 levels in plasma, significantly decreasing after 10-14 days of admission. No correlation with mortality was found. PTX3 expression in stained-tissue samples from patients was prominent around lung infection sites with influx of inflammatory cells including neutrophils and macrophages. Experimental melioidosis revealed increased PTX3 levels in murine plasma, bronchoalveolar lavage fluid, lung and liver samples over time. Histopathology of *B. pseudomallei*-infected mice tissues mirrored patterns observed in patient tissues.

Discussion:

Our findings demonstrate PTX3 as a potential novel biomarker for melioidosis. Although PTX3 was not associated to mortality in our cohort, classical expression patterns and infection severity during disease was reflected. Similar trends were observed during experimental melioidosis. In future studies, data from PTX3-knockout and pre-treated mice will aim to determine the functional role of this protein during experimental melioidosis and explore its therapeutic potential.

Implementation of a selective culture procedure for diagnosis of melioidosis reveals an increased number of cases in patients admitted to hospitals in north-central Vietnam

Trinh, Thanh Trung[a]; Nguyen, Quang Huy[b]; Nguyen, Phuong Anh[a]; Bui, Nguyen Hai Linh[a]; Que, Anh Tram[c]; Tran, Thi Le Quyen[a]; Mai, Van Tuan[d]; Vu, Thi Ngoc Anh[a]; Pham, Thị Huyen[e]; Do, Duy Cuong[b]; Michael, H. Norris[f]; Jason, K. Blackburn[h,i]

[a]Institute of Microbiology and Biotechnology, Vietnam National University, Hanoi, Vietnam; [b]Center for Tropical Diseases, Bach Mai hospital, Hanoi, Vietnam; [c]General Hospital of Nghe An province, Vietnam; [d]Hue Central Hospital, Hue, Vietnam; [e]General Hospital of Ha Tinh province, Vietnam; [f]Pathogen Analysis and Translational Health Group, School of Life Sciences, University of Hawai'i at Mānoa, Honolulu, Hawai'i, United States of America; [h]Spatial Epidemiology & Ecology Research Laboratory, Department of Geography, University of Florida, Gainesville, Florida, United States of America; [i]Emerging Pathogens Institute, University of Florida, Gainesville, Florida, United States of America

Although the bacterium *Burkholderia pseudomallei*, the causative agent of melioidosis, readily grows on routine media such as blood agar, MacConkey agar, and chocolate agar, diagnosis of the disease is challenging, particularly in non-sterile clinical specimens because of the overwhelming growth of other commensal bacteria. In this study, we implemented a selective culture procedure for screening patients suspected of melioidosis based on clinical signs at three large hospitals in north-central Vietnam. The procedure included enriching bacteria in selective broth and growing bacterial colonies on Ashdown agar. In this study, clinical specimens were collected from 5,027 febrile patients between September 2023 and June 2024. When using routine agar plates, *B. pseudomallei* was detected in 119 patients. When using the selective culture procedure, *B. pseudomallei* was detected in an additional 18 patients, which were confirmed using PCR. Laboratory findings and clinical features between two melioidosis patient groups were compared. Here, our study reports a high number of sepsis and respiratory infections as melioidosis with at least 137 confirmed cases among three provincial hospitals in under one year. This is due in part to expanded training and standardization of case definitions to care providers and implementing improved diagnostic tools. We confirmed an increased number of cases of melioidosis when implementing the selective culture procedure. This study provides guidance to improve case determination and diagnostics in areas of increased risk (due to habitat type and human activities; such as agriculture) and limited hospital resources.

Potential Mosquito-Associated Melioidosis and Analysis of Sample Processing Results in Hainan, China

Xun Kang; Shaowen Cheng; Rui Zheng; Hengjie Zhu; Qianfeng Xia

School of Tropical Medicine, Hainan Medical University, Haikou, China

We explored the possible transmission of *Burkholderia pseudomallei* (the pathogen responsible for melioidosis) through mosquito bites. A case study is presented involving a 31-year-old male patient in Hainan, China, who developed melioidosis following a mosquito bite. Laboratory analysis confirmed the presence of *B. pseudomallei* in the patient's wound. Subsequent environmental sampling detected the bacterium in a small number of Aedes albopictus mosquitoes. This study highlights the potential for mosquito-borne transmission of *B. pseudomallei* and calls for further research to confirm this transmission route.

In vitro activity and minimum inhibitory concentration of Epetraborole against *Burkholderia pseudomallei*

Amornchai, Premjit; Wuthiekanun, Vanaporn; Alley, MRK; Prior, Stephen; Day, Nicholas; Limmathurotsakul, Direk.

Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

Epetraborole (EBO) is a new antibiotic that inhibits leucyl-tRNA synthetase, essential for protein synthesis. EBO may be effective against *B. pseudomallei* and could be used as an adjunct to the standard antibiotic treatment for acute melioidosis; including ceftazidime (CAZ) or meropenem (MEM). This study examined EBO's *in vitro* activity and minimum inhibitory concentration (MIC) against *B. pseudomallei* using Thai isolates. We tested six *B. pseudomallei* isolates for frequency of resistance (FoR) with 4xMIC EBO and conducted a checkerboard assay on three isolates to see how EBO works when combined with CAZ or MEM. The fractional inhibitory concentration (FIC) index was used to assess synergy, additivity/indifference, and antagonistic effects between EBO and CAZ, and EBO and MEM. We determined MIC values for 90 clinical *B. pseudomallei* isolates using the broth microdilution (BMD) method according to CLSI guidelines. The resistance frequency in *B. pseudomallei* ranged from 1.57×10^{-8} to 9.85×10^{-8} CFU/mL. The MIC_{90} of EPT was 1 µg/mL, with a range of 0.5-4 µg/mL. EBO combined with CAZ or MEM showed no antagonistic activity. In conclusion, EBO is effective against *B. pseudomallei in vitro* with a low MIC value, and its combination with CAZ or MEM is additive/indifferent. The study supports further clinical evaluation of EBO.

A Novel Purified Bioactive Bovine Lactoferrin is Able to Inhibit the Growth of *Burkholderia* Species and Prevent Biofilm Formation

Xander, Christian[1]; Mlynek, Kevin[1]; Snedeker, Jonathan[2]; Hlubb, Christopher[2]; and Bozue, Joel[1]

[1]Bacteriology Division, U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID), Frederick, MD, USA, [2]Lactea Therapeutics, Frederick, MD, USA

Mammals rely on their innate immune system to protect against infectious diseases. One component of the innate immune system that helps combat these insults is the glycoprotein lactoferrin. Lactoferrin is known to exhibit broad spectrum activity against a multitude of bacteria, fungi, and viruses due to its multi-functional mode of action. Recently, Lactea Therapeutics and its affiliates have developed a novel, patent-pending technology to purify naturally derived bovine lactoferrin (Lactea Lf) as a medical countermeasure, not previously available, to ultra-high purity having retained its native multifunctional activities. In Lactea's studies, Lactea Lf demonstrated both inhibition of growth and elimination of biofilm formation against multiple nosocomial bacterial pathogens. To assess Lactea Lf as a medical countermeasure against biothreat pathogens, we generated dose-response curves against several select-agent bacteria and their respective BSL-2 surrogate strains including *Burkholderia psuedomallei*, *Burkholderia mallei*, and *Burkholderia thailandensis*. Here, we show that Lactea Lf can reduce the final culture density and inhibit growth in a dose dependent manner for all *Burkholderia* species tested. Of note, biofilm formed by *B. thailandensis* and *B. pseudomallei* was also reduced independent of growth inhibition, consistent with a multi-functional mode of action against these bacteria. Taken together, these data support that Lactea Lf is a promising new candidate for further studies as a broad-spectrum antimicrobial medical countermeasure with efficacy against several high priority biodefense-related bacterial pathogens. Based upon these data, future small animal studies will be pursued.

Disclaimers: The research described herein was sponsored by the Defense Threat Reduction Agency JSTO-CBD (project number CB11335).

The opinions, interpretations, conclusions, and recommendations presented are those of the authors and are not necessarily endorsed by the U.S. Army and Department of Defense.

Antibody-conjugated Polymersomes for Targeted Antibiotic Delivery to Intracellular *B. thailandensis*

Cheetham, Rebecca[1]; Taylor, Adam[2]; Newman, Tracey[1]; Lee, Seung[1]; Mahajan, Sumeet[1]; Whelan, Adam[2]; Evans, Nick[1]

[1]University of Southampton, United Kingdom; [2]DSTL-Porton Down, Salisbury, United Kingdom

Polymeric nanoparticles, polymersomes, may improve antibiotic delivery and clearance of difficult-to-treat, facultative intracellular *Burkholderia pseudomallei*. Polymersomes target bacteria, occupying the protective intracellular niche, by retaining antibiotic payload until internalisation by cells, however their targeting is not pathogen-specific. In this study, we hypothesised that antibody could be covalently linked to polymersomes and modifying polymersomes with *B. pseudomallei* anti-capsular polysaccharide monoclonal antibody (3VIE5) would enhance *Burkholderia thailandensis* (HG2 surrogate organism) interaction, improving inhibition of intracellular infection.

Aminated polymersomes, loaded with doxycycline, were fabricated by nanoprecipitation of 15 mg/mL polymer-DMF solution (PEO(5k)-*b*-PCL(18k) and H2N-PEO(5k)-*b*-PCL(17k), mass%=50/50) in aqueous antibiotic receiver solution. Dynamic light scattering measured nanoparticle size. Cross-linker, sulfo-SMCC, activated NH2-polymersomes were reacted to TCEP-reduced fluorescent antibody for 6-hours prior to dialysis and centrifugation. Fluorescence intensity (FI) was measured to assess conjugation. Coherent anti-stokes Raman scattering (CARS) tuned to the Raman CH-stretch region produced label-free, chemically informative images of RAW264.7 cells incubated with or without polymersomes.

Polymersomes were uniform and ≈100 nm in diameter. The FI of cross-linked polymersomes was greater than control suspensions, polymersomes omitting cross-linker and antibody-alone ($p<0.001$). The linear antibody-FI standard curve indicated a conjugated antibody concentration of 1.5 µg/mL. CARS imaging revealed greater signal from CH-stretch in cells treated with polymersomes compared to controls, suggesting intracellular polymersome uptake.

These data suggest sulfo-SMCC conjugates antibody to polymersomes. This methodology may now be used to fabricate *B. pseudomallei*-specific antibody-polymersome conjugates, assessing conjugation using ELISAs. Ongoing work will investigate the antimicrobial activity of the novel targeted treatment using *B. thailandensis in vitro* infection assays.

Assessing the abilities of Factor H-Fc IgG fusion protein variants as a therapeutic against *Burkholderia pseudomallei*

Hugo Sigona Gonzalez[1], Kelly Morgan[1], Irum Syed[1], Keith Wycoff[2], R. Mark Wooten[1]

[1]Department of Medical Microbiology and Immunology, The University of Toledo College of Medicine and Life Sciences, Toledo, Ohio 43614; [2]Planet Biotechnology Inc., Hayward, CA, USA.

Burkholderia pseudomallei (Bp) is a Gram-negative bacterium causing melioidosis, consequently leading to lethal sepsis and other severe pathologies. Bp is considered a Tier 1 select agent, and there is great interest in identifying targets for preventive therapeutics or those that ameliorate disease progression to better allow antimicrobial interventions. One key virulence mechanism is its ability to evade complement-mediated immunity. Our studies have determined that virulent Bp is naturally resistant to complement-mediated killing and treatments that promote complement-deposition allow efficient neutrophil-mediated opsonophagocytic killing. We have identified a Bp surface protein that binds host Factor H (FH), a negative regulator of the complement cascade, in its active form to promote immune evasion. Collaborating with Planet Biotechnology Inc., we have developed and are testing chimeric molecules with FH-binding sites fused to the constant (Fc) region of human immunoglobulin G(IgG), which should competitively inhibit FH-binding and protective activities by Bp, while the IgG Fc region activates the complement cascade for immune-mediated direct and/or opsonophagocytic killing. Preliminary studies show that a subset of these constructs binds Bp, initiates C3 deposition, and forms membrane attack complexes (MAC) on the bacterial surface. Additionally, we found that introducing a natural splice variant of FH, Factor H-related protein 1 (FHR-1), disrupts FH binding, enhancing complement deposition and killing by neutrophils. Current studies on second-generation constructs indicate improved Bp binding and complement activation, though direct bacterial killing was minimal. We are now evaluating their efficacy with opsonophagocytic killing via neutrophils and future studies will observe its protective potential in mice.

Should individuals receiving myelosuppressive anti-cancer chemotherapy in regions where melioidosis is endemic receive trimethoprim/sulfamethoxazole (TMP-SMX) prophylaxis?

Tej Shukla,[1] Simon Smith,[1] Kristoffer Johnstone,[2] Patrick Donald,[1] Josh Hanson.[1,3]

[1]Department of Medicine, Cairns Hospital, Cairns, Queensland, Australia; [2]Pharmacy department, Cairns Hospital, Cairns, Queensland, Australia; [3]The Kirby Institute, University of New South Wales, Sydney, NSW, Australia.

Background

Cancer increases the risk of melioidosis, but the characteristics and clinical course of melioidosis in patients with cancer has not been examined in detail.

Methods

All cases of culture proven melioidosis between 01/01/1998 and 01/06/2023 were examined. The characteristics and clinical course of patients with – and without – cancer were compared. The incidence of melioidosis in patients receiving anti-cancer chemotherapy who did – and did not – receive TMP-SMX was determined.

Results

An active cancer was present in 47/446 (11%) cases of melioidosis diagnosed during the study period; there was no association between melioidosis and any cancer type. Myelosuppressive chemotherapy had been prescribed to 14/47 (30%) in the 12 months prior to the diagnosis of melioidosis. Melioidosis was slightly more common in patients not receiving TMP-SMX chemoprophylaxis during their myelosuppressive chemotherapy than in those that did (15/3436 (0.44%) versus 1/652 (0.15%), number needed to treat: 345), but there was no difference in the incidence of fatal melioidosis between those that did and did not receive TMP-SMX chemoprophylaxis (5/3436 (0.15%) versus 1/652 (0.15%)). Twelve months after the diagnosis of melioidosis, 25/47 (53%) were still alive; 9/22 (41%) deaths were due to melioidosis and 13/22 (59%) were due to the underlying cancer.

Conclusions

Patients with cancer are predisposed to developing melioidosis and myelosuppressive chemotherapy increases this risk further. However, in this region of Australia, there was no difference in fatal melioidosis in patients who did – and did not – receive TMP-SMX chemoprophylaxis during their myelosuppressive anti-cancer chemotherapy.

Adverse reactions to trimethoprim/sulfamethoxazole for melioidosis eradication therapy: an evaluation of frequency and risk factors

Martin, Genevieve E; Bramwell, Joshua; Gadil, Eden; Woerle, Celeste; Ewin, Thomas; Davies, Jane; Janson, Sonja; Currie Bart J.

[1]Global and Tropical Health Division, Menzies School of Health Research, Charles Darwin University, Darwin, Northern Territory, Australia; [2]Infectious Diseases Department, Royal Darwin Hospital, Northern Territory, Australia; [3]Department of Infectious Diseases, University of Melbourne, Melbourne, Australia

Trimethoprim/sulfamethoxazole is the first-line agent for oral eradication therapy for melioidosis but has been associated with toxicity in this context. This study aimed to quantify adverse drug reactions (ADRs) to trimethoprim/sulfamethoxazole when used for treatment of melioidosis, and assess risk factors for ADR development.

A retrospective review of antimicrobial associated ADRs was performed in all patients treated for melioidosis in the Northern Territory of Australia from January 2017 – September 2022.

268 treatment episodes from 256 individuals were included. The frequency of clinician-attributed ADRs to trimethoprim/sulfamethoxazole (51% of exposed) was higher than for other antimicrobials used (ceftazidime 12%, meropenem 8% and doxycycline 12% of those exposed; $p<0.0001$). 44% of those treated with trimethoprim/sulfamethoxazole required drug cessation or dose reduction and 5 individuals (2%) had a severe cutaneous adverse reaction, with one fatality. Acute kidney injury was the most frequent ADR (25% of those exposed), with age (OR 1.04, 95% CI 1.02-1.06) and pre-existing renal disease (OR 5.11, 95% CI 1.57-16.5) independently associated with its development (overall $p<0.0001$).

Here we report very high rates of ADRs attributed to trimethoprim/sulfamethoxazole resulting in frequent discontinuation of this drug as part of oral eradication therapy for melioidosis. Further work is needed to balance the necessity and toxicity of this drug in this clinical context.

Establishing PK/PD drivers of therapeutic efficacy for *B. pseudomallei*: bridging *in vitro* and *in vivo* results using neutropenic mouse and hollow-fibre infection models

J. Matthew Meinig[1], Nathan B. Unsworth[2], Annette M. Gray[1], Jesmin Akter[2], Stephanie A. Halasohoris[1], Justin R. Doward[2], Ashley L. Babyak[1], Dianne W. Xu[2], Mary K. Hourihan[1]

[1]Bacteriology Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD, USA; [2]CBRN Defence, Sensors and Effectors Division, Defence Science and Technology Group, Fishermans Bend, Victoria, Australia.

Effective dosing of anti-infective therapeutics is dependent on understanding the pharmacokinetic/pharmacodynamic (PK/PD) relationship that drives antimicrobial activity. While robust *in vivo* PK/PD models are routinely used for pathogens in the public health space, appropriate models have not been reported for bacterial infections of biodefense concern including melioidosis. Here, we describe the use of both *in vivo* and *in vitro* models specifically to define PK/PD parameters associated with bacteriological success in *Burkholderia pseudomallei* infections. The murine neutropenic thigh infection (NTI) model is considered gold-standard for deriving PK/PD targets and is commonly evaluated by regulatory agencies for public health indications and directly informs clinical dosing decisions. Here, we report the first use of an NTI model using *B. pseudomallei* and have validated the model using standard-of-care. From this study, we have derived for the first-time *in vivo* PK/PD drivers specific for ceftazidime and *B. pseudomallei*. Additionally, the hollow-fibre infection model (HFIM) has been utilized to derive PK/PD parameters for *Burkhodleria* spp. *in vitro*. Results from the HFIM with surrogates of *B. pseudomallei* correlate well with outcomes in the murine NTI model. Importantly, these results together suggest that prior assumption on PK/PD targets for melioidosis based on other gram-negative pathogens may underestimate required PK/PD drivers leading to underdosing in future trials. This highlights the need to develop and utilize biodefense specific PK/PD models to inform dosing decisions for novel medical countermeasures.

Machine Learning based Genome Wide Association Study concerning antimicrobial resistance in *Burkholderia pseudomallei*

Phillips D. Paul[1], Hall M. Carina[1], Somprasong Nawarat[1], Schweitzer P. Herbert[1], Wagner M. David[1], Keim Paul[1], Mayo Mark[3], Currie J. Bart[3], Buscaglia Robert[2], Sahl Jason[1]

[1]Pathogen Microbiome Institute, Northern Arizona University, Flagstaff, Arizona, U.S.A.; [2]Northern Arizona University, Flagstaff, Arizona, U.S.A.; [3]Menzies School of Health and Research, Darwin, Australia

Although genome-wide association studies (GWAS) have been used for >17 years to uncover genotype/phenotype relationships in humans, it has only recently been used to explain bacterial phenotypes (e.g., virulence). In this study, we describe a novel, machine learning (ML) workflow used to identify novel mechanisms of antimicrobial resistance (AMR) in *Burkholderia pseudomallei*. Using minimum inhibitory concentrations (MICs) from a set of 274 isolates, we identified a S72F amino-acid mutation in the ambler domain of the *penA* β-lactamase enzyme associated with amoxicillin/clavulanate acid resistance. We then expanded the analysis to a set of 24 antibiotics tested across this set of *B. pseudomallei* isolates. Using raw MIC values, as opposed to categorical "resistant" and "susceptible" values, we identified novel AMR mechanisms across clinically-relevant drugs. Our ML workflow, which leverages modern algorithms and high-performance computing, allows for the robust identification of cryptic and associated AMR mechanisms. The implementation of novel ML algorithms for AMR detection will have direct implications for the early and effective treatment of deadly human pathogens, including *B. pseudomallei*.

Burkholderia pseudomallei Resistance Towards Co-trimoxazole in Sarawak: Is It a Concern?

Sumbu, Liana Lantong[1]; Sia, Tonnii[2]; Lal, Anand Mohan[3]; Ooi, Mong-How Ooi[4]; Podin, Yuwana[1].

[1]Institute of Health and Community Medicine, Universiti Malaysia Sarawak, Sarawak, Malaysia; [2]Miri Hospital, Miri, Sarawak, Malaysia; [3]Bintulu Hospital, Bintulu, Sarawak, Malaysia; [4]Sarawak General Hospital, Kuching, Sarawak, Malaysia.

Burkholderia pseudomallei is inherently resistant to numerous antibiotics and is the causative agent of the potentially fatal disease, melioidosis. Co-trimoxazole is a vital antibiotic in the eradication phase of melioidosis treatment. Of late, there have been emerging reports of higher minimal inhibitory concentration readings for co-trimoxazole among *B. pseudomallei* clinical isolates from Sarawak, Malaysian Borneo. The objectives of this study are to determine the prevalence and to understand the mechanism of such phenomena in Sarawak. The antibiotic susceptibility of *B. pseudomallei* clinical isolates collected from various hospitals in the Sarawak region was assessed using disk diffusion and E-tests. The susceptibility of the isolates against trimethoprim and sulfamethoxazole was further characterised using the broth microdilution method. The MIC breakpoints were analysed according to the CLSI and EUCAST guidelines. Overall, the Sarawak clinical *B. pseudomallei* isolates exhibited susceptibility of 96.3% (CLSI) and 97.6% (EUCAST) towards co-trimoxazole *in vitro*. Broth microdilution results suggested that several isolates were resistance to sulfamethoxazole and trimethoprim separately, which interestingly does not affect their susceptibility towards co-trimoxazole. Analysis of the MIC results reaffirms the significance of the CLSI guideline for antibiotic susceptibility testing and antimicrobial resistance surveillance in Sarawak. It is hoped that results from this study provide reassurance to clinicians in Sarawak that the use of co-trimoxazole is indeed effective for the treatment of melioidosis. Findings of this study warrants further characterization on the molecular and genetic basis of the isolates' resistance towards both sulfamethoxazole and trimethoprim.

The association between statin therapy and the subsequent clinical course of patients with melioidosis

Laura Prideaux,[1] Meg Sandeman, [1] Hayley Stratton,[1] Simon Smith, [1] and Josh Hanson.[1,2]

[1]Department of Medicine, Cairns Hospital, Cairns, Queensland Australia; [2]The Kirby Institute, University of New South Wales, Sydney, NSW, Australia

We examined all cases of culture-confirmed melioidosis in Far North Queensland, Australia since January 2017 to determine if statin therapy had any impact on patients' in-hospital clinical course. There were 100/321 (31%) taking a statin at the time of their melioidosis diagnosis. In univariate analysis, statin therapy was more common in patients with known macrovascular disease (odds ratio (OR) (95% confidence interval (CI)): 5.60 (3.17-9.90), p<0.0001), chronic kidney disease (OR (95%CI): 4.21 (2.16-8.21), p<0.0001) and diabetes (OR (95%CI): 2.81 (1.71-4.63), p<0.0001) and in older patients (OR (95%CI): 1.05 (1.03-1.06). A smaller proportion of patients taking statin therapy died before hospital discharge (5/100 (5%) versus 26/221 (12%); this difference did not reach statistical significance in univariate analysis (OR (95%CI): 0.39 (0.15-1.06), p=0.07). However, in multivariate analysis, while patients taking statin therapy were more likely to have macrovascular disease (OR (95%CI): 3.51 (1.86-6.65), p<0.0001) have diabetes (OR (95%CI): 2.62 (1.47-4.69), p=0.001) and be older (OR (95%CI): 1.05 (1.03-1.07), p<0.0001), they were less likely to die in hospital than patients not taking statin therapy (OR (95%CI): 0.23 (0.07-0.74), p=0.01). This statistical association does not necessarily prove that statin therapy reduces the case-fatality rate of melioidosis and most studies, including meta-analyses of randomized trials, show no benefit of statin therapy in patients with sepsis. However, the absence of any association between statin therapy and potential confounding factors (including gender, First Nations Australian status, remote residence or socioeconomically disadvantage) that might explain the observed association, means that the finding warrants further prospective examination.

DOES SUB-LETHAL EXPOSURE OF *BURKHOLDERIA PSEUDOMALLEI* TO ANTIBIOTICS INDUCE RESISTANCE?

F. Satyaputra* [1], P. Sivabalan [1], I. Gassiep [2], R. Norton [1]

[1]Pathology Queensland, Townsville University Hospital, Townsville, Queensland, Australia; [2]Pathology Queensland, Royal Brisbane and Women's Hospital, Brisbane, Queensland, Australia

Aim:

The objective of this study was to determine whether repeated exposure of *Burkholderia pseudomallei* isolates to increasing, sub-lethal doses of antibiotics used in the treatment of melioidosis selects for phenotypic resistance.

Background:

The treatment option for melioidosis is limited due to the inherent resistance of *Burkholderia pseudomallei,* the causative agent, to multiple classes of antimicrobials. Options include ceftazidime or meropenem for initial treatment followed by cotrimoxazole, doxycycline or amoxicillin-clavulanate for eradication. Resistance to these antimicrobials is rare but significant as it potentially leads to treatment failure. This study postulates that resistance can develop in isolates repeatedly exposed to sub-lethal antimicrobial concentration from either poor adherence or suboptimal dosing.

Methods:

Four isolates were included in this study: isolate A & C were fully susceptible whilst isolate B was resistant to ceftazidime, and isolate D to doxycycline and cotrimoxazole. Their identification was confirmed using BioMerieux Vitek2XL, and their baseline minimum inhibitory concentration (MIC) against meropenem, ceftazidime, cotrimoxazole, and doxycycline were obtained using gradient diffusion method (Etest BioMerieux). Serial doubling dilution of each antibiotics was performed on sterile tubes followed by inoculation of the isolates starting from the lowest antibiotic concentration. When growth occurred, indicated by turbidity, the isolates were sub-cultured to subsequent tubes with doubling antibiotic concentrations and to blood agar to check for purity. This process was repeated until the highest concentration of the antibiotic or where growth no longer occurred. The identification and MIC of isolates from these tubes were assessed and compared to the baseline.

Results:

We have shown that, with few exceptions, the MICs of the four isolates did increase up to 64 times the original MIC, when repeatedly exposed to increasing sublethal antibiotic concentrations. This applied to all antimicrobials commonly used in the treatment of melioidosis.

Isolates	Antibiotics	Baseline MIC (pre-exposure)	Highest MIC on exposure	Final Identification on Vitek2
A	Meropenem	0.5 µg/mL (S)	> 32 µg/mL (R)	*Burkholderia pseudomallei*
	Ceftazidime	1 µg/mL (S)	32 µg/mL (R)	*Burkholderia pseudomallei*
	Cotrimoxazole	1 µg/mL (S)	8 µg/mL*	*Pseudomonas aeruginosa / Pseudomonas fluorescens/ Sphingomonas spp.*
	Doxycycline	1 µg/mL (S)	64 µg/mL (R)	*Burkholderia pseudomallei*
B	Meropenem	4 µg/mL (S)	Unchanged #	N/A
	Ceftazidime	12 µg/mL (R)	> 256 µg/mL (R)	*Burkholderia pseudomallei*
	Cotrimoxazole	1 µg/mL (S)	Unchanged #	N/A
	Doxycycline	2 µg/mL (S)	48 µg/mL (R)	*Burkholderia pseudomallei*
C	Meropenem	1 µg/mL (S)	> 32 µg/mL (R)	*Burkholderia pseudomallei*
	Ceftazidime	2 µg/mL (S)	> 256 µg/mL (R)	*Burkholderia pseudomallei*
	Cotrimoxazole	2 µg/mL (S)	> 32 µg/mL (R)	*Burkholderia pseudomallei*
	Doxycycline	1 µg/mL (S)	64 µg/mL (R) *	*Pseudomonas aeruginosa / Pseudomonas fluorescens*
D	Meropenem	1 µg/mL (S)	> 32 µg/mL (R) *	*Pandorea spp.*
	Ceftazidime	1 µg/mL (S)	32 µg/mL (R)	*Burkholderia pseudomallei*
	Cotrimoxazole	> 32 µg/mL (R)	N/A ^	N/A
	Doxycycline	64 µg/mL (R)	N/A ^	N/A

No increased MIC

* Increased MIC but *B. pseudomallei* not confirmed as final identification

^ Unable to demonstrate higher MIC due to method limitation (baseline MIC is the highest MIC on Etest strip)

Conclusions:

This study showed that resistance in Burkholderia pseudomallei can be induced by sublethal exposure to antimicrobials used in in the treatment of melioidosis. This could have clinical implications if adherence is a problem. Molecular studies are required to evaluate the underlying mechanism of this finding.

DISCLOSURE: I would like to inform that this poster presentation had previously been presented in Antimicrobial 2023 Society's 22nd Annual Scientific Meeting at Brisbane Convention & Exhibition Centre, South Brisbane, Queensland, Australia

Meropenem resistant *Burkholderia pseudomallei* - A concerning single case in Australia with no prior meropenem exposure

Pirathaban Sivabalan, Ferris Satyaputra, Ian Gassiep, Brian Forde, Jaimie Frazer, Matthew Glover, Buenafe Adams, Robert Norton

[1]QML Pathology, Microbiology, Brisbane, Queensland, Australia; [2]University of Queensland, Brisbane, Queensland, Australia; [3]Department of Infectious Diseases, Logan Hospital, South Brisbane, QLD 4101, Australia.

Introduction

Burkholderia pseudomallei is a bipolar gram-negative bacillus found predominantly in soil of tropical regions of the world. The organism is the causative agent of melioidosis, a clinical disease with significant morbidity and mortality. It is inherently resistant to numerous antimicrobial agents. Resistance to carbapenems would therefore be of concern, given the limited treatment options. To date the primary resistance of B. pseudomallei to carbapenems is extremely rare.

Case Presentation

We report a case of cutaneous melioidosis in a 54-year-old male with poorly controlled type II diabetes mellitus. He was empirically treated with episodic doxycycline and trimethoprim-sulfamethoxazole, however the abscess re-accumulated. The patient had no prior exposure to meropenem. A sub-population of the isolate was meropenem resistant with an MIC>32 µg/L and the identification was re-confirmed as B. pseudomallei. Whole genome sequencing with ARDaP analysis only revealed resistance determinant to doxycycline. It did not reveal a resistance determinant to meropenem. The most plausible explanation is that the mechanism of resistance is not known and is not present in the ARDaP database. Furthermore, no carbapenemases were detected through the Abricate program, using both CARD and ResFinder databases.

Conclusion

To date this is the first reported case in Australia of a B. pseudomallei isolate resistant to meropenem without previous carbapenem exposure. Given the limited antibiotic options to treat this potentially fatal infection, understanding the mechanisms of resistance is important to help prevent future resistance. Hopefully in the evolving era of sequencing, more knowledge will be acquired about antimicrobial resistance within B. pseudomallei.

Interplay between outer membrane protein channels of the resistance nodulation cell division (RND) efflux pumps in *Burkholderia pseudomallei*

Somprasong, Nawarat[1]; Hagen, Johannah P.[1]; Settles, Erik W[1,2]; Keim, Paul S[1,2]; Schweizer, Herbert P.[1]

[1]The Pathogen and Microbiome Institute, [2]Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona, USA

The RND efflux pumps play critical roles in the intrinsic and acquired multidrug resistance in *B. pseudomallei*. Out of 10 *B. pseudomallei* RND systems, AmrAB-OprA, BpeAB-OprB and BpeEF-OprC have been characterized. Only AmrAB-OprA is expressed in wild-type strains and higher expression is achieved in *amrR* mutants. BpeAB-OprB and BpeEF-OprC are only expressed in regulatory mutants. In this study, we assessed the susceptibility profiles for AmrAB-OprA proficient and deficient strains in the *B. pseudomallei* Bp82 background. These included the *amrA*$^+$*B*$^+$-*oprA*$^+$ operon deletion [Δ(*amrAB-oprA*)] and an *oprA* deletion. As expected, this pump is the lone gentamicin resistance determinant in *B. pseudomallei*. Surprisingly, deletion of *oprA* alone in the wild-type *amrR* or the Δ*amrR* background did not increase gentamicin susceptibility to that observed in the Δ(*amrAB-oprA*) mutant. We investigated one possible scenario where the AmrA$^+$B$^+$ is still present in the Δ*oprA* mutants and might interact with an unknown outer membrane channel protein in the absence of OprA. By performing gene deletion, we discovered that in the absence of OprA, AmrA$^+$B$^+$ can function with OprB by forming an AmrA$^+$B$^+$-OprB$^+$ complex which can efflux gentamicin. Of note, OprB is normally associated with the BpeA$^+$B$^+$-OprB$^+$ efflux pump where it cannot extrude gentamicin. Our data suggest an interplay between outer membrane protein channels in antibiotic resistance mechanism in *B. pseudomallei*.

Wet-season Melioidosis Prophylaxis in Medical Oncology Patients at Royal Darwin Hospital over 5 Wet Seasons

[1]Tay, Sze yen; [2]Thompson, Chantelle; [3]Hsu, Kristin; [4]Celeste Woerle; [1,4]Meumann, Ella; [1,4]Currie, Bart; [5]Charakidis, Michail; [5]Karanth, Narayan

[1]Infectious Diseases Department, Royal Darwin Hospital, Darwin, Australia, [2]Department of Pharmacy, Royal Darwin Hospital, Darwin, Australia, [3]Department of Medical Oncology, Flinders Medical Centre, Adelaide, Australia [4]Menzies School of Health Research, Charles Darwin University, Darwin, Australia, [5]Department of Medical Oncology, Royal Darwin Hospital, Darwin, Australia

Introduction: In Darwin, NT Australia, the majority of melioidosis occurs in the wet season. Wet-season cotrimoxazole prophylaxis has reduced melioidosis in haemodialysis patients. A similar prophylactic strategy was introduced in mid-2017 for oncological patients.

Methods: A retrospective review was conducted to assess the efficacy of wet-season melioidosis prophylaxis (given November to April each year) in preventing melioidosis in oncological patients on active treatment over 5 wet seasons (Nov 2015 to April 2020). Since November 2017, prophylaxis was offered to patients having high-risk chemotherapy or high-dose steroids, or those with past melioidosis or positive melioidosis serology (indirect haemagglutination titre ≥ 1:40). Doxycycline was used as an alternative. Data were extracted from the Darwin Prospective Melioidosis Study and hospital records.

Results: 307 out of 1433 (21%) oncology patients received prophylaxis. Five patients were diagnosed with melioidosis (2/307 (0.7%) prophylaxis group vs 3/1126 (0.3%) non-prophylaxis group). An additional 17 melioidosis cases were found in oncological patients not receiving therapy for their malignancy or who had subsequent malignancy diagnosed on melioidosis workup. Of the 22 total cases, 11 had confirmed metastatic malignancy and 19 had another significant risk factor for melioidosis (9/19 had diabetes, 3/19 were pre-diabetic). Over the 5 wet seasons pre-and-post intervention, the proportion of patients on prophylaxis increased (6.2%, 8.9%, 15.5%, 32.0%, 35.2%), while the proportion of melioidosis cases declined [1.2% (3/242), 1.2% (3/259), 1.6% (4/251), 1.0% (3/303), 0.5% (2/378)]. Two deaths resulted from melioidosis, and 1 death was attributed to cotrimoxazole use (1/263).

Conclusion: Melioidosis in oncological patients is uncommon and most respond well to therapy for melioidosis. The target oncological population for wet-season prophylaxis needs to be better defined.

Evaluation of piperacillin-tazobactam dosing regimens for melioidosis treatment using the hollow-fibre infection model

Unsworth, Nathan; Akter, Jesmin; Doward, Justin; Xu, Dianne

CBRN Defence, Sensors and Effectors Division, Defence Science and Technology Group, Fishermans Bend, Victoria, Australia 3207.

Scientists have long noted that piperacillin-tazobactam (TZP) has potent activity against *Burkholderia pseudomallei*. Consequently, is has been suggested that in vivo or clinical studies to determine TZP effectiveness as a melioidosis treatment should be undertaken. However, the limited clinical information that has been published suggests that TZP may not be an effective treatment. Here we used the in vitro hollow fibre infection model (HFIM) to assess the effectiveness of TZP to treat a *B. thailandensis* infection, a good surrogate of *B. pseudomallei* in the HFIM. The HFIM is able to dynamically change the antibiotic concentration a bacterial population is exposed to, enabling precise mimicry of human antibiotic dosing and clearance over an entire treatment course. Accordingly, in nosocomial pathogens, HFIM results correlate well with clinical outcome. Using the *B. thailandensis* HFIM to conduct dose fractionation and escalation studies, the pharmacokinetic/pharmacodynamic (PK/PD) driver for TZP is likely Cmin/MIC. We also found that dosing TZP 3375mg q6d gave a superior treatment outcome than dosing q8d 4500mg using the recommended 30min IV infusion. To augment this data, Monte Carlo simulations were used to generate 1001 virtual human piperacillin PK profiles, which were interrogated to determine optimal TZP dosing. The population PK analysis suggests that extending the infusion time of TZP beyond the recommended 30min will increase the probability of obtaining a successful treatment outcome. This project demonstrates the strength of the HFIM, in being able to both assess antibiotic effectiveness and determine optimal dosing requirements of melioidosis therapeutics.

Impact of Geographical and Climatic Factors on Melioidosis Hotspots in Southern Taiwan

Chen Kuang-Yueh[a], Chen Chien-Cheng[b], Ren Chung-Yuan[a]

[a]Department of Physics, National Kaohsiung Normal University, Taiwan; [b]Department of Biotechnology, National Kaohsiung Normal University, Taiwan

Melioidosis, caused by *Burkholderia pseudomallei*, is usually clustered in the 5 km^2 melioidosis-hotspot at north of the hills; however, the reasons remain unclear. It is hypothesized that the hills, likely a barrier, resulted the phenomena to keep retention of the aerosols contaminated with *B. pseudomallei* during presence of northwesterly wind and rainfall, and further resulted the appearance of melioidosis case-clusters via people inhalation. Thus, the northern region of the hills defined a studied area while the southern area as the reference area. A total of 297 melioidosis cases, 48.64% of anti-flagellin antibodies in healthy individuals, and, approximately, 51~3067 copies of *B. pseudomallei*-specific *orf2* amplicons in the aerosols were found in studied area that significantly higher than those (159 cases, 9.2% of serum positivity and 2~16 copies of amplicons) in the reference area. The incidence of melioidosis cases was correlated with the concentration of the aerosols contaminated with *B. pseudomallei*, as well as related to the appearance of the rainfall, wind speed, and the times of wind northwesterly in the studied areas but no association was found in the area that without hill terrain. A Rouse equation prediction model, with 73% accuracy, figured out that aerosols at a height of 1.5m and over 0.75 m/s of wind speed can optimally envelope the studied area. Our results concluded that the hill features as well as rainfall, wind speed and wind direction, play a role in triggering the spreading of *B. pseudomallei* and formation of a melioidosis hotspot in southern Taiwan.

Presence of *Burkholderia thailandensis* in soil, Suriname, South America

Savelkoel, Jelmer[1]; Zimmermann, Rosalie; Soetosenojo, Ansmarie; Dzebisasjvili, Tsira; Lanzl, Maren; Matamoros, Sébastien; Mawie, Terrence; Woittiez, Lycke; Vreden, Stephen; Birnie, Emma; Wiersinga, W Joost

[1]Amsterdam UMC location University of Amsterdam, Amsterdam, the Netherlands

Background

Burkholderia pseudomallei (Bps) and *Burkholderia thailandensis* (Bth) have recently been identified in the environment of the United States. Previously, it has been postulated that slave trade introduced Bps from the African to the American continent. However, Bps and Bth have never been identified in Suriname, despite the presence of Bps in neighboring countries. The aim of this study was to identify Bps and Bth in Surinamese soil.

Methods

We performed a soil sampling study according to the consensus guidelines for isolating Bps. Samples were collected at a depth of 60-80 cm in cattle pastures, wasteland, and rice fields in northern Suriname. After enriched culture, morphologically suspected colonies were subjected to oxidase and antimicrobial susceptibility tests. We performed PCR for Bps/Bth on all presumptive isolates and confirmed positive findings with WGS.

Results

Out of 400 soil samples collected, we identified zero Bps, but four Bth isolates confirmed by both PCR and WGS. All Bth isolates were from a single rice field in the district Nickerie in northwestern Suriname. We next compared the four Bth isolates to other sequenced Bth isolates and found the Surinamese isolates closely matched to other American and African isolates.

Conclusions

For the first time, we found Bth in soil of Suriname and South America. The genomic relatedness to other American and African isolates can support the theory that Bth might also have been introduced through former slave trade routes. Although Bps was not found in Suriname, this does not necessarily preclude its presence and might be due to limited sampling.

Centrifugation to detect *Burkholderia pseudomallei* in turbid river water

Zimmermann, Rosalie; Krenz, Juliane; Robinson, Matthew T; Pierret, Alain; Ribolzi, Olivier; Xayyathip, Khampaseuth; Vongsouvath, Manivanh; Dance, David

Department of Medical Microbiology, Amsterdam University Medical Centers, Amsterdam, The Netherlands

Knowledge of the presence and abundance of *B. pseudomallei* in rivers is important to understand the environmental distribution and dispersal of this pathogen that causes melioidosis, a neglected infectious disease in tropical regions. However, the often highly turbid river water limits the amount of water that can be filtered, which makes detection and quantification difficult, especially if *B. pseudomallei* loads are low.

We tested centrifugation as an alternative method to filtration in order to concentrate larger amounts of suspended particles for the detection of *B. pseudomallei* in river water samples from the Mekong river in Vientiane, Laos, between the rainy and dry season in 2023. The centrifugation sludge and filters from river water and centrifugation supernatant filtration were cultured on Ashdown agar, and DNA was extracted after enrichment and directly for targeted qPCR according to established protocols. We additionally spiked river water samples with *B. pseudomallei* to test both methods with defined *B. pseudomallei* concentrations.

Preliminary results showed that *B. pseudomallei* was present during the whole sampling period but detected variably in different sample types without a clear advantage of larger water samples. Interestingly, the centrifugation supernatant was more frequently positive than the centrifugation sludge. The particle size distribution of the suspended sediment before and after centrifugation is currently being analyzed. As expected, qPCR after enrichment was the most sensitive method to detect *B. pseudomallei* with the disadvantage that (semi-)quantification is not possible. The results of the spiking experiments may help to understand the limits of the tested methods.

Missing links of melioidosis in India: A cross-sectional analysis of case reports, agrometeorological and socioeconomic factors.

Jha, Shivvrat: Mittal, M.; Prasad, LR; Dastidar, SG; Shetty, S; Mailapalli, DR; Dastidar, RG; Mukhopadhyay, C; Lal, Piyush.

Center for Emerging and Tropical Diseases, Department of Microbiology, Kasturba Medical College Manipal, Manipal Academy of Higher Education, Manipal, Udupi, India

Melioidosis, an emerging tropical infectious disease and a global threat, lacks a robust disease prediction model owing to the suboptimal information related to the incidences and associated factors. This article focusses on spatial data analysis of melioidosis patients in India, a tropical country considered to be endemic for the disease. We analyzed case reports of 1694 patients from 1953 until December 2023 against agrometeorological and socioeconomic factors. Our findings suggest people of all ages being susceptible to the disease with higher prevalence among farmers, age ranging between 40 to 55 years and with long standing diabetes mellitus. Interestingly, despite the multiple favorable factors for bacterial growth across India, cases were reported mostly in southern India suggesting the under-reporting, under-diagnosis or misdiagnosis of the disease. Though incidences were higher during wet season, data lacks correlations to create a robust regression model. Main outcome of this study is a "melioidosis checklist index" for efficient case reporting and emphasis on the need to strengthen data points at regional-level by creating extensive awareness among susceptible populations, physicians and para-medicals globally. The enriched data of case reports and associated factors will help formulate public health policies in near future.

Efficacy of Chlorination Against *Burkholderia pseudomallei* Sequence Types ST-1996 and ST-70 Implicated in a 2022 Urban Melioidosis Outbreak in Hong Kong

WONG Tak-Fai; CHOW Wang-Ngai Franklin; SIU Kit-Hang Gilman

Department of Health Technology and Informatics, The Hong Kong Polytechnic University, Hong Kong Administrative Region, People's Republic of China

Melioidosis outbreaks have historically been associated with inadequately chlorinated water, with effective chlorination previously managing the spread of *Burkholderia pseudomallei*. In 2022, Hong Kong experienced an urban melioidosis outbreak, prompting government suspicions that insufficient chlorination facilitated *B. pseudomallei* transmission from soil to residential areas via drinking water. This outbreak, characterized by sequence types ST-1996 and ST-70, resulted in a 3-4 fold increase in annual cases concentrated in one district. However, the efficacy of chlorination against these specific sequence types remained unknown.

This study evaluated chlorine disinfection against these sequence types by testing the effects of different free chlorine levels, temperature, and pH levels under controlled laboratory conditions. At 0.5ppm, a 6-log reduction required 30 minutes, while 1.0-1.5ppm achieved this within 3 minutes. Besides chlorine level, disinfection efficacy increased significantly with higher temperatures, and lower pH promoted efficacy by maintaining a higher proportion of HOCl. Additionally, no *B. pseudomallei* was successfully recovered after a 0.5ppm 60-minute exposure in all conditions, indicating no chlorine-tolerant persisters.

To better reflect real-world conditions, testing was also conducted using portable water samples from service reservoirs. Pre-chlorination of the reservoir water resulted in improved disinfection efficacy compared to laboratory-generated water. Our results demonstrated that exposure to 0.5ppm chlorine for 10 minutes achieved over 4-log reduction against ST-1996 and ST-70, indicating that adherence to WHO standards of maintaining a minimum 0.5ppm residual chlorine level in potable water would provide effective protection against these outbreak-associated sequence types. These findings provide evidence to guide policymakers in setting chlorination levels that protect public health by mitigating waterborne transmission of *B. pseudomallei*. Further studies should evaluate local environmental sequence types to establish comprehensive safeguards against melioidosis.

Top End Troubles: Addressing the Financial Strain of Melioidosis Care in the Northern Territory of Australia

Smith, Emma; Marshall, Catherine; Annesley, Kirsty; de Graaff, Barbara; Currie, Bart; Davies, Jane

Menzies School of Health Research, NT, Australia

Background:

Melioidosis is endemic in the Top End of Australia. In the Northern Territory 60-100 cases of melioidosis are diagnosed each year, many of which require hospitalization for prolonged periods. Hospital funding in Australia is set at a national level, based on the average cost for each Diagnostic Related Group (DRG). Given the complexities of melioidosis and it's limited regional distribution, this average cost does not reflect the true costs incurred in this setting.

Methods:

A retrospective review was conducted including all patients hospitalized with confirmed melioidosis in the Top End of the NT in the financial year 2021-2022. Data were extracted from the National Hospital Cost Data Collection (NHDC), and from hospital medical records for admissions within that same year. NHDC data were examined to assess if the correct DRG had been assigned to each admission. Total cost of each admission was calculated and compared to funding received by the health service.

Results:

There were 64 patients admitted to Top End NT hospitals with melioidosis from July 2021-June 2022. The estimated total cost of melioidosis admissions over this period was $6,219,274 (AUD). Length of stay was variable with a median of 34 days (Range 2-107 days). Re-admission for melioidosis-related issues was common (37.5% of patients). Most patients were assigned the appropriate DRG code "Other Infectious and Parasitic Diseases". The total variance in funding was -$1,085,012.59 (AUD). Positive variance was more common in those admitted to ICU and ventilated. The highest negative variance was in those with bone and joint melioidosis.

Conclusion:

Despite hospital admissions with melioidosis being coded to the most appropriate DRG, the annual deficit was more than one million dollars. It is likely a new DRG is required encompassing Top End complex tropical infections.

Paediatric melioidosis with erythema nodosum-like cutaneous manifestations

Nerida Moore, Alexandra Groves, Robert Duguid, Joshua Francis, Te-Yu Hung, Jennifer Yan

Background: Melioidosis is caused by Burkholderia pseudomallei (BPS) and is a rare and difficult to diagnose infection in the paediatric population, accounting for only 5% of all cases in the Northern Territory where it is endemic. Erythema nodosum is a relatively common dermatological manifestation of several infectious and autoimmune conditions. We describe four children in the Northern Territory of Australia with rashes clinically, but not histologically, consistent with erythema nodosum (EN) who after thorough investigation were diagnosed with melioidosis.

Cases: All four cases were previously well children with no significant co-morbidities. All presented with acute, painful and erythematous lower limb nodules that were clinically diagnosed as EN after dermatology review. Two of the four cases had skin biopsies; case 1 had histological findings consistent with dermatitis and case 2 had non-necrotizing granulomatous inflammation consistent with infection (BPS not isolated on tissue). Only case 4 had culture confirmed melioidosis at a site of clinical infection, however all four cases had very high IHA titres suggestive of melioidosis and case 1 and 3 isolated BPS on throat and rectal swabs. Case 1 and 2 had pulmonary nodules on imaging that were presumed to be secondary to melioidosis though were asymptomatic of these with no clinical history of severe pulmonary infections. All four cases achieved clinical cure of their EN and though case 4 was lost to follow up after intensive phase therapy.

Conclusion: Rarely EN may occur as a manifestation of melioidosis and melioidosis should be suspected in paediatric cases of EN in BPS endemic settings.