2 SAMC

2nd South Asian Melioidosis Congress 2017

Unearthing a Subterranean Infection

29-30 August 2017

Organising Committee

- Patron: Prof Vasanthi Thevanesam (Sri Lanka)
- Chairperson: Dr Enoka Corea (Sri Lanka)

- Prof Chiranjay Mukhopadhayay (India)
- Prof Tim Inglis (Australia)
- Prof David Dance (Laos)
- Dr Herbert Schweizer (US)
- Dr Dharshan de Silva (Sri Lanka)
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- Dr Malika Karunaratne (Sri Lanka)
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Message from Patron

Prof. Vasanthi Thevanesam, Emeritus Professor of Microbiology, University of Peradeniya

Louis Pasteur, in 1854, made the oft quoted remark translated 'In the fields of observation, chance favours only the prepared mind'. The unveiling of the beginning of the story of melioidosis in Sri Lanka echoes this truth at different levels.

The presentation of a woman in her early 60's with a temporal abscess, to a hospital with a developing microbiology service and an off-site microbiologist in 2006, with the very recent availability of internet access in the University of Peradeniya allowing immediate access to information and advice, led to collaboration with Prof Tim Inglis and a research study by Dr Enoka Corea which led to the unearthing of a neglected tropical disease – melioidosis – in Sri Lanka.

The hosting of the 2nd South Asian Melioidosis Congress in Sri Lanka is one of the outcomes of this story. As those who work in resource limited countries, it is a reminder that working together and using whatever resources we have makes it possible to achieve beyond our wildest dreams.

My congratulations to Dr Corea, Prof Inglis and all others who contributed so much time, energy and resources towards the unraveling of this story. May this conference be an encouragement, a source of expanding collaboration and add greatly to the store of knowledge about this disease in the countries in which it takes its toll.

Message from Chief Guest

Dr Razia Pendse, WHO Representative to Sri Lanka

Melioidosis or Whitmore's disease is a bacterial infection acquired through direct contact with contaminated soil and water, through broken skin, inhalation and close contact.

Sri Lanka lies in the melioidosis belt. Largely a hidden disease, increasing number of cases are being reported from the country. Increased frequency of natural disasters like floods, landslides etc. may increase the risk of infection. There are not many prevention biomedical interventions. Vaccine is in trial phase. Diagnosis requires a high degree of suspicion and early diagnosis is the key to reduce morbidity and mortality as we have effective antibiotics.

The World Health Organization and Sri Lanka College of Microbiologists enjoy a healthy, collaborative partnership and it is a great honor to be part of the 2nd South Asian Melioidosis Congress.

I thank the organizers for inviting WHO to the meeting and I wish all the participants fruitful and academically enriching deliberations and discussions.

Message from Chairperson, 2nd SAMC

Dr Enoka Corea, President, Sri Lanka College of Microbiologists

The journey continues……..The 2nd South Asian Melioidosis Congress is a follow up to the ground breaking effort of the team at Kasturba Medical College in Manipal to initiate a regional meeting on melioidosis, bringing together global experts and doctors, microbiologists, scientists and researchers from South Asia to exchange ideas and learn from each other.

Melioidosis remains a 'neglected' disease, so neglected that it is not even recognized as one by the WHO! South Asia is predicted to have the largest burden of disease but much of this burden remains hidden and unrecognized. Hence, the theme of this conference, "Unearthing a subterranean infection".

The theme also emphasises the source of the causative bacterium, *Burkholderia pseudomallei*. In the tropical countries of South Asia, a large proportion of the population are engaged in subsistence agriculture, often walk barefoot and use natural source of water for drinking and bathing. Therefore, exposure to *B. pseudomallei* is ubiquitous and infection is seen in all strata of society and encompasses all age groups. Disease becomes a manifestation of underlying immune compromise.

The South Asian Melioidosis Congress is an effort to shine a spotlight on melioidosis in India, Sri Lanka, Bangladesh, Nepal, Bhutan and Pakistan. The disease is frequently mis- or under-diagnosed in the region and revealing the hidden burden of infection requires the co-operation of a network of microbiologists, epidemiologists, infectious disease specialists, and public health personnel. The Congress will allow researchers and clinical personnel meet and learn from global experts on the disease and develop collaborations within and between countries in the region and groups overseas with the ultimate goal of reducing the morbidity and mortality of this potentially fatal infection.

The objectives of the congress are to raise awareness among clinicians, public health personnel, policy makers and the public of the burden of disease caused by melioidosis in South Asia, to disseminate knowledge and skills pertaining to melioidosis within the South Asian region, to promote basic and applied research in melioidosis and to promote collaboration among clinicians and researchers in the region to study the epidemiology, clinical and laboratory diagnosis, management and prevention and control of melioidosis.

We hope that the South Asian Melioidosis Congress will join the World Melioidosis Congress and the European Melioidosis Congress as a "must attend" event in the calendar of "melioidistas" *(melioidista = those who are 'bitten by the bug' melioidosis).*

2nd South Asian Meliodosis Congress - 2017

PROGRAM SCHEDULE

Tuesday 29th August, 2017

8.00 - 9.00am Registration and Inauguration			
Speakers	**Affiliation**	**Lecture Topic**	**Time**
Prof. David Dance	University of Oxford, UK	Historical background of melioidosis	9.00 - 9.30am
Dr. Direk Limmathurotsakul	Mahidol University, Bangkok, Thailand	Global epidemiology of melioidosis	9.30 - 10.00am
Prof. Chiranjay Mukhopadhyay	Kasturba Medical College, Manipal University, India	Epidemiology of melioidosis in South Asia	10.00 - 10.30am
10.30 - 11.00am Tea Break			
Dr. Enoka Corea	University of Colombo, Sri Lanka	Nationwide epidemiology of melioidosis in Sri Lanka	11.00 - 11.30am
Prof. Md Shariful Alam Jilani	Ibrahim Medical College, Dhaka, Bangladesh	Epidemiology of melioidosis in Bangladesh	11.30 - 12.00noon
Dr. Apichai Tuanyok	University of Florida, USA	One health initiative to uncover epidemiology of melioidosis in southern Thailand	12.00 - 12.30pm
Dr. Mohan Natesan	Division of Molecular and Translational Sciences, USAMRIID	Host and pathogen specific biomarkers for melioidosis disease management	12.30 - 1.00pm
1.00 - 2.00pm Lunch Break			
Dr. Dharshan de Silva	General Sir John Kotelawala Defense University	Immune responses in *B. pseudomallei* infection	2.00 - 2.30pm
Prof. Natkunam Ketheesan	Editor of the book, *Melioidosis - A Century of Observation and Research*	Fatal attraction: host-pathogen interactions in *B. pseudomallei* infection	2.30 - 3.00pm
Prof. Joost Wiersinga	University of Amsterdam, Netherlands	Insights into the pathogenesis of *B. pseudomallei*	3.00 - 3.30pm
Dr. Ganjana Lertmemongkolchai	Khon Kaen University, Thailand	Mechanisms of human susceptibility to *B. pseudomallei* infection	3.30 - 4.00pm
4.00 - 5.00pm Case Presentations 5.00 - 5.30pm Tea Break			

Wednesday, 30th August, 2017

8.00 - 9.00am Meet the Expert			
Speakers	**Affiliation**	**Lecture Topic**	**Time**
Prof. Tim Inglis	Path West Laboratory, Australia	The clinical correlates of barefoot bacteraemia	9.00 - 9.30am
Dr. K. E. Vandana	Kasturba Medical College, Manipal University, India	Septicaemic melioidosis: The best approach to diagnose	9.30 - 10.00am
Dr. Narisara Chantratita	Mahidol University, Bangkok, Thailand	Laboratory diagnosis of melioidosis	10.00 - 10.30am
10.30 - 11.00am Tea Break			
Dr. T. A. K. Chaitanya	Kasturba Medical College, Manipal University, India	Molecular epidemiology of *B. pseudomallei* in South Asia	11.00 - 11.30am
Mr. Adam Merrit	PathWest Laboratory, Australia	Molecular epidemiology of *B. pseudomallei* in Sri Lanka	11.30 - 12.00noon
Dr. Herbert P. Schweizer	University of Florida, Emerging Pathogens Institute, USA	Antibiotic resistance mechanisms in *B. pseudomallei*	12.00 - 12.30pm
Dr. Wirongrong Chierakul	Mahidol University, Bangkok, Thailand	Update on treatment of melioidosis	12.30 - 1.00pm
1.00 - 2.00pm Lunch Break			
Prof. Ivo Steinmetz	Medizinische Universität Graz Österreich	Update on soil surveillance	2.00 - 2.30pm
Mr. Tushar Shaw	Kasturba Medical College Manipal, India	Soil epidemiology of melioidosis in SW India	2.30 - 3.00pm
Prof. Tim Inglis	PathWest Laboratory, Australia	'Omics' of *B. pseudomallei*	3.00 - 3.30pm
3.30 - 4.00pm Valedictory 4.00 - 4.30pm Tea Break			

Historical background of melioidosis

Prof. David Dance is a Senior Clinical Research Fellow/ Consultant Microbiologist, Lao Oxford Mahosot Hospital Wellcome Trust Research Unit, Lao PDR/Centre for Tropical Medicine, University of Oxford, UK and a Honorary Professor, Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, UK.

Melioidosis was first recognised by Alfred Whitmore and his assistant CS Krishnaswamy working in Rangoon, Burma (now Myanmar) in 1911. The first two decades of research on Whitmore's bacillus was dominated by British workers in Burma and the Federated Malay States, particularly Stanton and Fletcher at the Institute for Medical Research in Kuala Lumpur, who first coined the term 'melioidosis'. During the 1920s and '30s, the disease was found also to be present in other British, French and Dutch colonies in Asia, with the first case in the Indian sub-continent reported from Sri Lanka in 1927. The demonstration that *Burkholderia pseudomallei* was present in the environment by researchers in French Indochina in the 1930s and 1950s completely changed our understanding of its epidemiology. From the 1940s-1970s the known global distribution was gradually extended, with the first cases from India reported in 1953, Australia in 1949, Thailand in 1955 and Bangladesh in 1964. The disease came to prominence as a cause of infection amongst French and American troops during the Vietnam conflicts, and began to attract attention from both the USA and USSR as a potential bioweapon around that time. The later designation of the organism as a Select Agent in the USA has given welcome impetus (and additional funding) to research into all aspects of melioidosis. During the 1980s, the true burden of melioidosis in countries in SE Asia began to become apparent, enabling a series of clinical trials to be undertaken in Thailand that have defined modern therapeutic regimens. The publication of the first *B. pseudomallei* genome sequence in 2004, and the rapid development of genomics, have created opportunities for rapid progress in our understanding of the basic biology, epidemiology and pathogenesis of melioidosis, as well as vaccine development. However, if the burden of melioidosis, which is thought to infect at least 165,000 people and cause 89,000 deaths per year worldwide, the brunt of which is thought to be borne by the Indian sub-continent, is to be reduced, the key will be to increase awareness of the disease, extend the coverage of basic microbiology services, and develop simple public health programmes that reduce exposure of vulnerable people such as diabetics to infection.

Global epidemiology of melioidosis

Direk Limmathurotsakul has been the Head of Microbiology at Mahidol-Oxford Tropical Medicine Research Unit (MORU), Mahidol University (http://www.tropmedres.ac), since January 2012. He also holds a Wellcome Trust Intermediate Fellowship in Public Health and Tropical Medicine

Introduction: *Burkholderia pseudomallei*, a highly pathogenic bacterium that causes melioidosis, is commonly found in soil in Southeast Asia and Northern Australia. Melioidosis can be difficult to diagnose due to its diverse clinical manifestations and the inadequacy of conventional bacterial identification methods. The bacterium is intrinsically resistant to a wide range of antimicrobials, and treatment with ineffective antimicrobials may result in case fatality rates exceeding 70%. The global distribution of *B. pseudomallei* and burden of melioidosis, however, remain poorly understood.

Objective: Here, we map documented human and animal cases, and the presence of environmental *B. pseudomallei*, and combine this in a formal modelling framework to estimate the global burden of melioidosis.

Methods: A globally comprehensive database was compiled, and a boosted regression tree (BRT) statistical model was used to estimate environmental suitability for *B. pseudomallei* globally at a resolution of 5 km × 5 km. We then used a multivariable negative binomial regression model and logistic regression model to relate the environmental suitability values generated by the BRT model to estimate the numbers of cases and deaths caused by melioidosis in each 5 km × 5 km square, respectively.

Results & Discussion: We estimate there to be 165,000 (95% credible interval 68,000-412,000) human melioidosis cases per year worldwide, of which 89,000 (36,000-227,000) die. We predict that only 40% of all melioidosis cases occur in the Southeast Asia and Pacific region, where melioidosis is considered highly endemic. By contrast, South Asia is predicted to bear 44% of the overall burden, because large populations live in areas contaminated with *B. pseudomallei*. We found that high rainfall and temperature, and anthrosol soil type (a type that has been modified profoundly by irrigated agriculture) were strongly associated with the presence of *B. pseudomallei*. Our estimates suggest that melioidosis is severely underreported in the 45 countries in which it is known to be endemic and that melioidosis is likely endemic in a further 34 countries which have never reported the disease.

Conclusion: The large numbers of estimated cases and fatalities emphasise that the disease warrants renewed attention from public health officials and policy makers.

Epidemiology of Melioidosis in South Asia

Dr. Chiranjay Mukhopadhyay is a Professor in the Department of Microbiology, Kasturba Medical College, Manipal, India. He is an EC member of the International Melioidosis Society (IMS). He hosts the Indian Melioidosis Research Forum (www.melioidosisindia.com), which has researchers from 12 states of India. He was the Chairperson of the 1st South Asian Melioidosis Congress in India in 2015.

Melioidosis was first reported from Rangoon (Burma), a South Asian country, by Whitmore and Krishnaswamy in the year 1912, from a 40 year old patient who died from an 'undescribed infective disease, somewhat resembling but really easily distinguishable from glanders'. Sixteen years later, it was diagnosed in a European tea broker from Sri Lanka, the country which is holding the 2nd South Asian Melioidosis Congress this year. This disease remained unrecognized for another 40 years, till the 1960s, before its endemicity was established in South Asian Countries like Malaysia, Singapore and Thailand. It took another 20 more years to get the first case of melioidosis diagnosed from India, the largest South Asian country which has recently been predicted to be the 'hot spot' of the disease. A journey of almost 100 years which started from gharry ponies has been completed to genome sequencing of the causative agent of 'Tapanuli fever' or 'the black corruption of Formosa' in the year 2004.

Thailand did not feature in the early description of the disease; however, interest and awareness was stimulated in late 1980s and early 1990s with inspired collaboration and a productive program when east met west. Unfortunately, this effort remained restricted to Thailand and a few other SEA countries like Laos PDR and Vietnam but failed to encourage researchers from countries like India, Bangladesh, and Sri Lanka. In recent years, however, there is increasing recognition of melioidosis in the Indian subcontinent, where networking of researchers gave rise to significant enthusiasm to diagnose more cases and inspired in-depth research. Nonetheless, it is not enough – more collective and collaborative effort and funding opportunities for South Asian researchers are needed to explore the 'unknown territories' of this neglected, tropical and extremely fatal disease which should culminate into development of preventive strategies for better public health.

Nationwide Epidemiology of Melioidosis in Sri Lanka

Dr. Enoka Corea is a Senior Lecturer at the Department of Microbiology, Faculty of Medicine, University of Colombo, Sri Lanka. She has spent over a decade unravelling the epidemiology of melioidosis in Sri Lanka and has shown it to be highly endemic in the country. She is currently the President of the Sri Lanka College of Microbiologists and is the Chairperson, 2nd South Asian Melioidosis Congress.

Melioidosis has been compared with an iceberg, since the majority of cases are hidden. National surveillance was instituted with a network of microbiology laboratories and a standard case definition, laboratory work up procedure and questionnaire. Primary isolation relied on conventional culture. Suspected isolates were referred to the reference laboratory for bacteriological identification and confirmation by real time PCR assays for LpxO. Demographic and clinical data of culture positive patients were analysed.

A total of 250 cases were recorded between 2006 and May 2017. Number increased annually with 103 (41%) detected in 2016. Males predominated, 179 (71.6%) being male. Sixteen were children. The age range was wide (2-92y) reflecting ubiquity of exposure to soil in the population. Commonest age group was middle-aged (41-60y), corresponding to likelihood of soil exposure and age of onset of diabetes. Majority (201/250, 80%) lived in rural areas. All provinces were affected with the highest number from the Western (n=88), North Western (n=54) and Eastern (n=33) Provinces. There were no cases in the cooler hill country where the main crop is not rice, but tea/rubber. Eighty seven patients presented between May/July and 81 between November/January, the two monsoonal periods (67%) with an increase in case load in June/July 2016 after heavy rains and flooding in May. Only 44 patients were farmers. There was representation of every population group. While diabetes was the predominant risk factor (n=163, 65.2%), organ disease, alcohol excess and thalassaemia were also predisposing factors. Melioidosis was seen in healthy adults and children (33/250, 13.2%). Clinical presentations included community acquired sepsis and pneumonia, superficial and deep abscesses and septic arthritis. Central nervous system and genitourinary infection was seen. One had endocarditis. Mortality was 20.4% (51/250).

The majority of isolates belong to the YLF clade but 38 strains belonged to the BTFC (Australian) type A total of 108 isolates were genotyped and 46 sequence types (STs) were identified, 40 being novel at the time of submission. ST1137 was commonest ST in Sri Lanka (n=18). Sri Lanka has the largest representation of all the South Asian countries in the international *B.pseudomallei* database, accounting for more than 2.4% of the entire database. The large number of STs (46) suggests that microevolution is occurring through genetic rearrangement. Melioidosis is endemic in Sri Lanka with a wide geographic and demographic distribution. Improved diagnosis has led to reduced mortality. There is an urgent need to extend surveillance of melioidosis to under-resourced parts of the country and to populations at high risk.

Epidemiology of melioidosis in Bangladesh

The recent work of Dr. Jilani, Professor of Microbiology, Ibrahim Medical College, Dhaka, demonstrated that a substantial number of Bangladeshis are exposed to *B. pseudomallei* and also identified for the first time the presence of this organism in the soil of Bangladesh. MLST of isolates revealed that four novel sequence types of *B. pseudomallei* exist in the Bangladesh environment.

The pioneering work of Indian bacteriologist C.S. Krishnaswami and British pathologist Alfred Whitmore first identified the organism *Burkholderia pseudomallei*, among Burmese morphine addicts in 1911. Since then, it took a century to determine its source in the environment of the Indian sub-continent. The organism was recovered for the first time in 2011 from the soil of Gazipur District of Bangladesh.

However, the first case of melioidosis from Bangladesh was reported in 1964 in a 29 year old British sailor who was travelling through Bangladesh and stayed in Chittagong for 3 months. Since then, melioidosis has been sporadically detected in Bangladesh over last several decades. The first melioidosis case in a native Bangladeshi child was diagnosed in 1988. Subsequently, five more cases were detected in U.K. among Bangladeshi immigrants from the Sylhet region from 1991 to 1999. Later on, at least 35 culture-confirmed melioidosis cases were detected among the diabetic patients in Bangladesh and all of these cases were diagnosed at Ibrahim Medical College and BIRDEM Hospital in Dhaka from 2001 to 2016.

So, it is evident that melioidosis is prevalent in Bangladesh. In order to determine the magnitude of exposure, two sero-epidemiological studies were conducted to elucidate the extent of melioidosis in the Bangladeshi population. In 2012, a hospital based serological survey using the indirect haemagglutination assay reported a 28.9% sero-positive rate among patients attending several tertiary care hospitals for unrelated ailments. The other study, conducted in 2013, used sonicated whole-cell antigen to determine anti *B. pseudomallei* IgG antibody by ELISA and detected a sero-positivity rate of 21.48% among healthy people attending the rural health care facilities of four northeastern districts of Bangladesh.

Analysis of the geographical distribution of melioidosis cases indicates that the disease is potentially endemic in eleven districts of the country. Out of these districts seven are located in the north and northeast region while the remaining four districts are located in the southern part of Bangladesh. As the majority of the cases were from northeastern districts of the country (like, Mymensingh, Gazipur, Sylhet and Dhaka) we consider those districts as the major endemic areas for melioidosis in Bangladesh. The disease is found to be highly seasonal with the majority of cases presenting during the rainy season (April to August), presumably because during this period the chance of exposure to the organism is highest.

The clinical presentation of melioidosis among Bangladeshi patients ranges from localized to systemic infection. However, no single clinical feature was found to be typical of melioidosis,

though fever was present in 100% of cases. The most frequent presenting features include acute fulminant septicemia to a chronic debilitating localized infection characterized by abscess formation in different organs of the body. Although more than 50% cases presented with focal abscesses, non-suppurative infection such as septicemia, arthritis, UTI and pneumonia were also prevalent. In Bangladesh diabetes has been documented as the most commonly associated concomitant risk factor found in >95% of cases of melioidosis. Out of 35 melioidosis cases admitted at BIRDEM hospital, 6 (18%) patients died. Most of these cases presented with septicemia and died within 24-48 hours of hospital admission and before the diagnosis of melioidosis was made. This stresses the need for early diagnosis and initiation of appropriate treatment.

Phylogenetic analysis of 22 clinical and 2 environmental isolates of *B. pseudomallei* by multi locus sequence typing (MLST) revealed thirteen different sequence types (STs), of which 4 STs (ST- 1352, 1124, 761 and 756) were novel types. All the strains containing ST 56 were isolated from patients with septicemia. ST 56, present in 5 clinical isolates, was the most common variant present in Bangladesh, followed by ST 1007 (4 cases) and ST 1005 found in 2 clinical and 2 soil isolates. Presence of ST 1005 in the soil of Gazipur district as well as its presence in melioidosis patients from the same area indicated soil as the source and reservoir. Gene cluster analysis targeting *Yersinia*-like fimbrial (YLF) and *B. thailandensis*-like flagellum and chemotaxis (BTFC) gene demonstrated that all 24 isolates contained YLF gene cluster. None of the isolates was positive for BTFC gene cluster. YLF is found predominantly in the South Asian region. Apart from the four novel STs described above, all other STs that have been detected in Bangladesh are also present in Thailand, Cambodia, China and Vietnam and other neighboring countries. It is likely that human and/or animal trafficking between these areas played a major role in the dissemination of *B. pseudomallei*. However, further analysis with a wider range of isolates from these regions is required to confirm the source and distribution of the organism in the region.

One health initiative to uncover epidemiology of melioidosis in southern Thailand

Dr. Apichai Tuanyok, a native of Thailand, received his Ph.D. in Tropical Medicine from the University of Liverpool, England in 2000. He has been working on most aspects of *Burkholderia pseudomallei* and its disease, melioidosis, since 2002 when he was a postdoc in Dr. Donald Woods' laboratory in Calgary, Canada. His current position is Assistant Professor of Medical Microbiology in the Department of Infectious Diseases and Immunology, College of Veterinary Medicine, University of Florida, USA.

Melioidosis is endemic in most parts of Thailand. However, the prevalence of melioidosis in humans and animals and the occurrence of its pathogen, *B. pseudomallei*, in the natural environment of southern Thailand have not been updated for long time. Our goal is to develop a "One Health" initiative for melioidosis investigation and promote multidisciplinary melioidosis research in southern Thailand. As a part of this initiative we have been collecting *B. pseudomallei* isolates from human and animal cases and soils in Songkhla Province since January 2014. All isolates from patients admitted to three tertiary care hospitals including Songklanagarind Hospital, Hatyai Medical Center, and Songkhla Provincial Hospital were sent to a melioidosis laboratory at Prince of Songkla University for species confirmation. Molecular diagnostics using real-time PCR such as TTS-1, BTFC&YLF, and LPS typing assays were used to identify *B. pseudomallei*. In addition, we have investigated the presence of *B. pseudomallei* in soils, especially in goat farms and a local zoo where animal cases have been reported. We used standard soil culturing techniques with selective media, Ashdown's agar and TBSS-50 broth, for *B. pseudomallei* isolation. Suspected bacterial colonies grown on Ashdown's agar were subjected to further identification by latex agglutination, lateral flow immunoassay (LFI) and real-time PCR. The project is ongoing and so far we have confirmed at least 158 melioidosis cases from humans as well as the presence of *B. pseudomallei* in soils in Songkhla and nearby provinces. The infections were mostly seasonal and associated with rainfall. Genetic analysis using multi-locus sequencing typing (MLST) has indicated that most of these recent isolates had the same STs as those from Finkelstein's historic collection from southern Thailand a half century ago. Specifically, strains with STs 288, 1323, and 1359 were frequently found in Songkhla. Interestingly, at least 6 patients were confirmed to be infected by more than one sequence type. This suggests a high genetic diversity of *B. pseudomallei* in natural sources. Strains with ST3 were found in human and animal cases as well as in the environment. Collectively, we believe that our "One Health" initiative of melioidosis would form an integral part of regional threat assessment of Thailand and Southeast Asia.

Host and pathogen specific biomarkers for melioidosis disease management

Dr. Mohan Natesan is an investigator at the Division of Molecular and Translational Sciences, United States Army Research Institute of Infectious Diseases, Frederick, Maryland, USA. Dr. Natesan obtained Ph.D. from All India Institute of Medical Sciences, India and post-doctoral training at Harvard Medical School, USA. Dr. Natesan studies antibody-target interactions in bacterial and viral diseases, using high-throughput methods, discovery of infection biomarkers, and specializes in assay miniaturization.

Fluctuations in the composition or abundance of analytes in biological fluids can provide valuable information for diagnosis of infection and monitoring disease progression. Towards this purpose, our laboratory has developed a proteomics workflow for identifying lead biomarkers of melioidosis. First, we utilized a multiplexed microarray constructed with more than 300 recombinant proteins derived from *Burkholderia mallei*, *B. pseudomallei* and *B. thailandensis*. Recombinant *Burkholderia* proteins (>300) were produced by *E. coli* expression system. The proteins were printed on nitrocellulose-coated slides using an ink-jet printer. The arrays were probed with serum samples from non-human primates (NHPs) exposed to *B. mallei* and human melioidosis cases. Elevated antibody responses to specific bacterial proteins were observed in post-exposure serum samples compared to pre-exposure or controls. Further analysis of the microarray results revealed several candidate pathogen proteins that are potential biomarkers of immune responses. We also used imaging mass spectrometry (iMS) in tandem with laser-capture microdissection and LC-MSMS to identify candidate biomarkers that were present in tissues from primate disease models. The primary MS data were obtained from standard formalin-fixed and paraffin-embedded tissue sections that were processed for optical microscopy. Host and pathogen proteins that were significantly perturbed by infection were identified by comparisons with control tissues. Calprotectin (CALP), a calcium and zinc binding protein, was elevated in serum of NHPs infected with *B. mallei*, and additional host biomarkers were also enriched within infected tissues. We further examined correlations between CALP and antibiotic treatment outcomes of melioidosis patients. Serum levels of CALP and C-reactive protein (CRP) were significantly higher in melioidosis and non-melioidosis sepsis patients compared to healthy controls. Median CALP levels were higher in melioidosis compared to non-melioidosis sepsis patients, whereas CRP levels were similar in both cases. Notably, intensive intravenous antibiotic treatment of melioidosis patients resulted in lower levels of CALP and CRP ($p<0{\cdot}0001$), coinciding with recovery. The median percent reduction of CALP and CRP was 71% for both biomarkers following antibacterial therapy. For acute melioidosis compared to sepsis caused by other infections, the area under the curve (AUC) for CALP was 0·75, and 0·57 for CRP, indicating that CALP is a better prognostic marker for melioidosis compared to CRP. Thus, reductions in serum CALP levels were linked to therapeutic responses to antibiotics. Our results demonstrate that a combination of multidimensional proteomics approach can be used to identify lead biomarkers of infection detectable in serum.

Immune responses in *Burkholderia pseudomallei* infection

Dr. Aruna Dharshan De Silva is currently Senior Lecturer and heads a Biomedical Laboratory at the Faculty of Medicine, General Sir John Kotelawala Defense University, Sri Lanka. He holds an Adjunct Associate Professor position at the La Jolla Institute of Allergy & Immunology, California, USA. Dharshan has been involved in melioidosis research since 2014 and has other research programs studying dengue and tuberculosis in Sri Lanka.

Melioidosis is a life-threatening infectious disease caused by the Gram-negative bacillus *Burkholderia pseudomallei*, predominantly found in southeast Asia and northern Australia. Our group has setup an extensive surveillance system in Sri Lanka over the last few years and identified increasing numbers of cases. Overall, we have screened ~3156 patient samples using in-house IHA testing for high levels of antibodies since mid-2014. A total of 171 positive cases were detected up to date (~5.4 % of all suspected cases). Multiple projects were carried out with these samples and a portion was used for the following studies.

The aim of this study was to establish useful correlation with disease biomarkers, comparing healthy individuals, patients with melioidosis and patients with sepsis caused by other pathogens, by analyzing gene expression levels of important cytokines. The study population consisted of 55 melioidosis cases, 20 healthy controls and 20 sepsis cases caused by other pathogens. A Qiagen common human cytokines array profiling the gene expression of 84 important cytokines were analyzed by real time quantitative PCR (RT-qPCR). Further, gene expression profiles of 25 gene targets including 19 immune response genes and 6 epigenetic factors using total RNA extracted from peripheral blood mononuclear cells (PBMC's) of study subjects were also analyzed.

Results of the first part showed consistently upregulated expression of interleukin (IL)-4, IL-17A, IL-23A, IL-24, IFNA1 and IFNB1, TNF superfamily 4 (TNFSF4), transforming growth factor (TGF), TGF beta 1, superfamily, bone morphogenetic proteins 3 and 6 (BMP3 and BMP6), and other growth factors such as macrophage colony-stimulating factor (M-CSF), C-fos induced growth factor (FIGF), and platelet-derived growth factor alpha (PDGFA) polypeptide, in melioidosis patients compared to their expression in other sepsis cases regardless of comorbidities, duration of fever/clinical symptoms, and antibiotic treatment. This suggests a dominant Th2- and Th17-type-cytokine response indicating their important role in disease pathogenesis though they were found to be dysregulated at initial stages of infection. IL-1A, IL-1B, and IL-8 were significantly downregulated in septicemic melioidosis patients compared to other sepsis cases. Thus, these differentially expressed genes may serve as biomarkers for melioidosis diagnosis and to understand immune response mechanisms.

The second set of experiments show Inflammatory response genes; TLR4, late onset inflammatory mediator HMGB1, genes associated with antigen presentation; MICB, PSMB2,

PSMB8, PSME2, epigenetic regulators; DNMT3B, HDAC1, HDAC2 were significantly down regulated, whereas the anti-inflammatory gene; IL4 was up regulated in melioidosis patients compared to sepsis cases caused by other pathogens. Septicaemic melioidosis cases showed significant down regulation of IL8 compared to sepsis cases caused by other pathogens. HMGB1, MICB, PSMB8, PSMB2, PSME2, HDAC1, HDAC2 and DNMT3B showed consistent down regulation of gene expression in melioidosis patients compared to other sepsis infection, irrespective of comorbidities such as diabetes, duration of clinical symptoms and antibiotic treatment.

This work is currently being expanded to decipher the specific pathways to understand how *B. pseudomallei* affects these human gene expression patterns

Fatal attraction: host-pathogen interactions in *B. pseudomallei* infection

Natkunam Ketheesan completed his high school education in Sri Lanka. After obtaining an MD from the Vinnitsa National Medical University, he went on to complete an MSc and PhD in immunology at the University of Leeds. He worked as a Research Fellow in the University of Leeds, University of Western Australia and the University of Queensland before accepting an academic position at an University in Australia. His research efforts focus on investigating the interactions between selected tropical bacterial pathogens and the human host.

Introduction: In some regions, individuals with type 2 diabetes (T2D) are more than ten times over represented among patients with melioidosis. In melioidosis, the severity of the infection and clinical outcomes are determined primarily by the presence or absence of host risk factors such as T2D. Our understanding of the mechanisms underlying susceptibility of individuals with T2D toward *Burkholderia pseudomallei* infection is limited.

Objectives: Our objective was to determine the early immune responses following *B. pseudomallei* infection in comorbid T2D.

Methods: To investigate the mechanisms underlying the increased susceptibility of individuals with T2D to *B. pseudomallei* infection, a relevant model of T2D-infection comorbidity was established. The diet induced murine model of T2D reflects the major features of T2D in human. Our studies were conducted by infecting the animals by different routes of infection using both virulent and less virulent strains of *B. pseudomallei*.

Results & Discussion: The initial systemic inflammatory cytokine response (TNF-α, MCP-1 and IL-12) was delayed in T2D mice during the first 12 hrs of infection and by 24 hrs post-infection *B. pseudomallei* loads were significantly higher in spleen, liver and lung in T2D compared to non-diabetic animals. Following an initial delay in cytokine production, an exaggerated proinflammatory cytokine response was observed in T2D mice by 48 hrs post-infection. T2D mice were highly susceptible to *B. pseudomallei*, with a median survival of 4 days compared to 12 days for non-diabetic mice. Experimental findings suggest that defects in the early immune response to *B. pseudomallei* infection significantly contribute to the greater susceptibility in T2D. Decreased phagocytic and antimicrobial capacity of neutrophils, dendritic cells and macrophages during the initial stages of infection impact on the downstream immunoregulatory functions, predisposing the diabetic host to clinically apparent infection. These dysregulated early responses may also lead to ineffective T cell and protective immune responses.

Conclusions: Findings in the last few years attribute the failure to mount a robust early immune response to *B. pseudomallei* infection as the fundamental cause of increased susceptibility of individuals with diabetes to this infection.

Pathogen interactions in *B.pseudomallei* infection

Professor Joost Wiersinga is chair of the Division of Infectious Diseases of the Academic Medical Center, University of Amsterdam. He received his medical training at the University of Amsterdam with additional courses at the Mayo Clinic (Rochester, Minnesota) and the National Institutes of Health (NIH, Bethesda, MD). He divides his time between patient care, teaching and research in the Center for Experimental Molecular Medicine (CEMM) all at the Academic Medical Center, Amsterdam.

This overview talk will summarize current understanding on the molecular characterization of *B. pseudomallei* and the immunology of melioidosis.

The genome of *B. pseudomallei* is composed of two chromosomes of which the largest part represents the *B. pseudomallei* core genome, whereas the remaining accessory genome has been associated with bacterial virulence. Virulence factors, most notably quorum sensing, type III secretion system, lipopolysaccharide and other surface polysaccharides, flagella and various factors essential for the intracellular life cycle of *B. pseudomallei*, have been further characterized.

The neutrophils play a critical in host defense, which is initiated by the Toll-like receptors. The proinflammatory immune response – including the activation of coagulation – and its regulation have been further dissected.

In summary, severe melioidosis can probably be seen as the clinical manifestation of a pathogen recognition receptor mediated dysregulation of the immune response to invading *B. pseudomallei*. *B. pseudomallei* employs numerous tactics to evade the immune response. Studies on host–pathogen interactions in melioidosis have identified a whole range of potential new treatment targets.

Mechanisms of human susceptibility to *Burkholderia pseudomallei* infection

Dr. Ganjana Lertmemongkolchai has spent the past 20 years working on neglected tropical diseases, specifically melioidosis, and more broadly sepsis and diabetes mellitus. Her core expertise is in cellular immunology, and her laboratory at Khon Kaen University in Thailand underpins major collaborative studies in human cellular immunology and biomarker research, including human signatures in expression profiling, neutrophil functions and large scale T and B cell epitope discovery.

Introduction : *Burkholderia pseudomallei* infection (melioidosis) is an increasing public health burden in NE Thailand and severely under-reported in other countries worldwide. This organism is a significant bio-threat agent and a facultative intracellular pathogen. Type 2 diabetes mellitus is the most common risk factor for melioidosis.

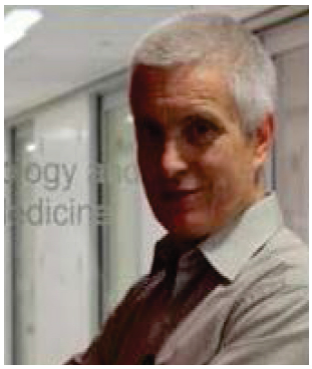
Objectives : To characterise human immune responses *to B. pseudomallei* in healthy persons versus patients with diabetes mellitus living in an endemic area of melioidosis.

Methods : Human neutrophil functions were studied by means of phagocytosis, killing by oxidative burst, migration, apoptosis and cytokine productions.

Results & Discussion : Human neutrophil responses were impaired in individuals with diabetes mellitus. More than half of the diabetic patients were prescribed glibenclamide (also known as glyburide) to control blood glucose levels. Recent evidence demonstrated that glibenclamide reduced pro-inflammatory cytokine production and migration capacity by neutrophils of diabetic individuals in response to this bacterial infection. Furthermore we found that glibenclamide decreased free glutathione levels and glutathione peroxidase of neutrophils after exposure to live *B. pseudomallei*. Interestingly exogenous glutathione could restore these functions.

Conclusion : Taken together, our data show a link between the effect of glibenclamide on glutathione and neutrophil functions in response to *B. pseudomallei* that may contribute to the susceptibility of diabetic individuals to *B. pseudomallei* infection.

The clinical correlates of barefoot bacteraemia



Prof Tim Inglis is a Professor of Pathology at the School of Pathology and Laboratory Medicine, University of Western Australia and Consultant Medical Microbiologist, Department of Microbiology, PathWest Laboratory Medicine, Perth, Western Australia where his team uses systems biology methods to study melioidosis. Recently completed projects include a US government-funded project on *Burkholderia pseudomallei* in which Prof Inglis was the lead investigator for the biogeographic attribution subproject.

Melioidosis is a single infection with a range of syndromic outcomes, ranging from rapidly fatal septicaemia with or without pneumonia, to localised internal organ or soft tissue infection. Five main clinic-pathological disease categories have been described; septicaemia with no, one or multiple foci, and localised focal or multifocal infection without septicaemia (S0, SS, SM, FS, FM, respectively).

In Sri Lanka the common occurrence of lower limb infection with or without septicaemia has been attributed to a barefoot lifestyle, linked to farming, gardening and other activities that put unprotected feet in contact with broken ground. The likely progression from bacterial inoculation through minor cuts and grazes to infection of the lower limbs and eventual bacteraemia presents opportunities for prevention, early diagnosis and improved treatment.

The cutaneous, abscess and joint infection variants of localised melioidosis appear to be under-represented in the national survey data. Increased recognition coupled with effective treatment may halt progression to septicaemic disease. Awareness of the bacteraemic consequences of a barefoot lifestyle needs to be raised through community health education

Septicemic melioidosis: Best approach to diagnosis

Dr. K. E. Vandana is a Professor at the Dept of Microbiology, Kasturba Medical College, Manipal, India. She was a recipient of the Fogarty Fellowship to attend the Sparkman Center Summer Institute for Global Health at the UAB School of Public Health, USA - 2009, July.

Myriad of clinical presentations and difficulties faced with laboratory diagnosis often delay the diagnosis of melioidosis. This has led to higher mortality of up to 50% in resource constrained settings, whereas early diagnosis and better sepsis management reduces the mortality to 10%. The gold standard for the diagnosis of melioidosis is culture from clinical specimens. This is, however, time-consuming and may not be easily available in endemic regions. Simple, rapid and reliable diagnostic tests for melioidosis will help identify cases earlier, leading to improved outcomes, given the delay of culture and identification techniques. The diagnostic yield in melioidosis often relies on the nature of clinical specimen and the corresponding bacterial load, blood containing lowest CFU/ml and respiratory secretions such as sputum with higher bacterial load.

We analysed 200 cases of laboratory confirmed melioidosis including 57 (29%) that presented with features of sepsis. Of these 57 cases, 45 (79%) were bacteremic. Additional 12 patients presented with deep organ abscesses and pulmonary melioidosis. Nearly 50% of patients of total cases of bacteremic melioidosis had features of sepsis. The mean time to positivity was 28.3 ± 9.03 hrs. EDTA blood specimen from a subset of the bacteremic cases were tested for PCR and all were negative while all the blood culture fluids were positive.

We also analysed the usefulness of different culture methods and PCR in the diagnosis of melioidosis, emphasizing on those patients whose blood cultures are negative. Clinical specimens (n=525) obtained from patients presenting with clinical symptoms suggestive of community-acquired pneumonia, lower respiratory tract infections, superficial or internal abscesses, chronic skin ulcers and bone or joint infections were tested for the presence of *Burkholderia pseudomallei* using conventional culture (CC), enrichment culture (EC) and PCR. Detection rates of *B. pseudomallei* using CC, EC and PCR were 3.8%, 5.3% and 6% respectively. Diagnostic sensitivities and specificities of CC and PCR were 71.4, 98.4% and 100 and 99.4% respectively in comparison with EC as the gold-standard test. An increase of 1.6% (95%CI: 1.08-4.32%) in the case detection rate of melioidosis was observed in the study population when EC and/or PCR were used in adjunct to the conventional culture technique.

In conclusion, blood cultures provide better diagnostic yield when the patients present with features of sepsis. However, given that good number of localized melioidosis also manifest with sepsis, access to tissue specimen and performance of enrichment culture and / PCR would increase the diagnostic yield.

Laboratory diagnosis of melioidosis

Narisara Chantratita is an associate professor at the Department of Microbiology and Immunology, Faculty of Tropical Medicine Mahidol University in Bangkok. She has studied the interactions between the pathogen and human host in melioidosis for over twenty years, combining laboratory investigation with clinical studies. She has conducted or been involved in numerous studies relating to melioidosis and other bacterial infections in northeast Thailand.

Introduction: Diagnosis of melioidosis is dependent on bacteria culture which is time-consuming and has low sensitivity. Indirect hemagglutination (IHA) is a widely used antibody detection method for melioidosis, but has a short-shelf life and is unstandardized and unreliable.

Objective: To obtain an effective target antigen for use in a simple point-of-care test (POC).

Methods: Rapid ELISAs using crude *B. pseudomallei* antigen preparations or purified O-polysaccharide (OPS), capsule, flagellin and hemolysin co-regulated protein (Hcp1) were compared using serum samples from three large collections obtained from melioidosis patients and patients with other bacterial infections.

Results: We detected high levels of antibodies to Hcp1 and OPS in serum from melioidosis patients upon admission and showed that anti-Hcp1 levels declined post-recovery. When serum samples from endemic areas were tested, the performance of the Hcp1-ELISA and combined Hcp1/OPS-ELISA were higher than the OPS-ELISA. When serum from non-endemic areas was tested, the combined Hcp1/OPS-ELISA gave the highest performance. Both the OPS- and Hcp1-based ELISAs were useful for detection of antibodies in various groups of patients including diabetics.

Conclusions: Since anti-Hcp1 titers in melioidosis patient serum were higher than anti-OPS titers, Hcp1 is an attractive candidate for further development of a rapid POC test for use in endemic areas.

Molecular epidemiology of *B.pseudomallei* in South Asia

Dr. Chaitanya Tellapragada is currently working as an Assistant Professor at the Department of Virus Research, Manipal University, Manipal, India. He is a medical microbiologist by training. He completed his Masters and Doctoral studies from the Department of Microbiology, Kasturba Medical College, Manipal. His primary area of research focuses on studying the molecular epidemiology and genetic determinants for antimicrobial resistance among bacterial pathogens.

Burkholderia pseudomallei, the etiological agent of melioidosis, is gaining recognition as an important cause for community-acquired bacteremia and pneumonia worldwide. While the disease is known to be highly endemic in Thailand and Northern Australia, there is mounting evidence from the published literature suggesting the possibility of melioidosis being endemic in South Asian countries such as India, Sri Lanka and Bangladesh. Nevertheless, protean clinical manifestations and the lack of awareness among microbiologists and clinicians restrict our understanding regarding the real burden of this disease in the South Asian countries.

Molecular epidemiology of *B. pseudomallei* in South Asia remains far from well-elucidated. Among the various molecular epidemiological tools available for studying the genetic relatedness/diversity among bacterial species, multi locus sequence typing (MLST) is a reliable, reproducible and cost-effective tool. Till date, MLST results of 223 isolates reported from South Asia countries (comprising 109, 78, 33 and 4 isolates each from Sri Lanka, India, Bangladesh and Pakistan respectively) are available on the MLST database https://pubmlst.org/bpseudomallei/. Genetic diversity, both at inter- and intra-national levels are high among the *B. pseudomallei* isolates in this region. STs 1137 and 1368 are the predominant genotypes reported from Sri Lanka and India respectively.

In our attempts to further elucidate the molecular epidemiology of melioidosis in India, we used MLST of clinical and environmental *B. pseudomallei* isolates obtained from distant geographical locations in southern part of the sub-continent and compared their genetic diversity with the isolates from rest of the world. Indian *B. pseudomallei* STs were genetically diverse from those of Australasian and South East Asian STs. There was no significant association between the isolated ST and the site of infection, clinical manifestations and/or the outcomes. Further, O-Lipopolysaccharide diversity was studied among the Indian isolates (N=213) and LPS-B (71%) was the most predominant form observed. Among the virulence determinants, presence of Bim A Bp was observed in more than 90% of the isolates.

Molecular epidemiology of *B. pseudomallei* in Sri Lanka

Adam Merritt is a Senior Scientist at PathWest Laboratory Medicine in Western Australia and is an expert in laboratory automation, molecular diagnostics for melioidosis and the biographic attribution of strains of *Burkholderia pseudomallei*. He is working towards his PhD on the phylogeography of *B. pseudomallei* in Western Australia.

B. pseudomallei isolates obtained from melioidosis cases in Sri Lanka from 2006 to 2016 were subjected to MLST. From 108 isolates 46 sequence types were obtained. Using this typing scheme the Sri Lanka melioidosis population is most closely related to isolates from South-Central Asia.

Drawing together soil type, rainfall and elevation data it was found that most cases are associated with haplic arisol soils and tend to occur at low altitudes in regions with moderate amounts of variation between the wet and dry season.

We previously described a new eBURST group of predominantly Sri Lankan isolates. While the cluster still exists the majority of the newer isolates cluster elsewhere in the eBURST tree. However, all but one appears in the section of the tree dominated by isolates from Australia. Maximum likelihood trees group the isolates into 7 clusters. Of these, groups 1 and 3 to 5 have fairly limited geographic distributions but groups 2 and 6, the most diverse, have broad geographic distributions.

In contrast to the possibility of particular STs being more common due to differential virulence we explored the idea that some STs may have been introduced in a more recent, non-geological timeframe. To explore this we placed the data in a historical context by colouring cases/isolates in the maximum likelihood tree according to the historical territory where they occurred. Examining three historical periods (Early Kingdoms, Transitional and Dutch colonial) we observe very little correlation with the Early Kingdoms period but increasing correlation with the latter two periods. We believe that group 6 isolates and possibly group 2 likely represent predominantly indigenous or regional strains of B. pseudomallei while isolates from the other groups may have originated from sea trade at regularly interspersed sea ports on the west and south coasts of Sri Lanka that were used at the time.

Addition of new clinical and environmental isolates and the transition to whole genome sequencing will allow this and other hypotheses to be fully tested.

Antibiotic Resistance Mechanisms in *Burkholderia pseudomallei*

Dr. Herbert P. Schweizer is a Preeminence Initiative Professor of Microbiology at the University of Florida and a member of the University's Emerging Pathogens Institute. His laboratory is a leader in the world for *Burkholderia pseudomallei* research and he has been instrumental in elucidating drug resistance mechanisms in this bacterium. In 2006, he was elected as a Fellow of the American Academy for Microbiology. He has served on and chaired grant evaluation and advisory panels in the areas of antimicrobial resistance and drug discovery research.

Antimicrobial resistance in Gram-negative bacteria has reached crisis status in many parts of the world. Although *Burkholderia pseudomallei* possesses drug resistance mechanisms commonly found in other Gram-negative bacteria[1], resistance is still curiously rare in this bacterium, but can occur in response to drug exposure. This presentation will discuss: 1) how *B. pseudomallei* employs diverse strategies to maximize exploitation of its chromosomally-encoded resistance determinant reservoir to acquire resistance in the absence of externally acquired resistance genes; and 2) how laboratory studies on resistance can inform treatment strategies for treatment *B. pseudomallei* infections (melioidosis) and diagnosis of resistant bacteria. The discussion will focus on resistance determinants that compromise use of antibiotics used for acute phase therapy (ceftazidime, amoxicillin + clavulanic acid, carbapenems) and eradication phase therapy (trimethoprim + sulfamethoxazole [co-trimoxazole or SXT] and doxycycline)[2].

Ceftazidime resistance (CAZ^r) is mostly mediated by the PenA β-lactamase[1]. This enzyme is a penicillinase, but has weak intrinsic cephalosporinase and carbapenemase activity. Overproduction of PenA as a consequence of promoter mutation or gene amplification leads to lower-level, but clinically significant resistance[3,4]. High-level CAZ^r is due to mutation of critical PenA amino acid residues or by a combination of such mutations with the promoter mutation or gene amplification[3,4,5]. CAZ^r as consequence of the penicillin-binding protein 3 target deletion has also been documented[6]. Clavulanic acid resistance is the result of a critical amino acid substitution in PenA[1]. Carbapenem resistance is very rare but has recently been documented in an Australian clinical isolate with a novel amino acid substitution in PenA[7]. Trimethoprim (TMP) and co-trimoxazole (SXT) resistance is primarily due to extrusion of the drugs by the BpeEF-OprC efflux pump[8]. Constitutive expression of this pump in *bpeT* mutants leads to TMP^r, but not SXT^r. The latter is caused by constitutive overexpression of BpeEF-OprC in *bpeS* mutants. BpeT and BpeS are closely related LysR-type regulatory proteins. Aside from efflux, mutations affecting the TMP target FolA and the folate biosynthetic pathway-associated FolM protein also contribute to resistance to TMP and sulfamethoxazole, respectively. BpeEF-OprC is the main doxycycline resistance determinant, but a ribosomal RNA modification enzyme and possibly increased outer membrane permeation have also been noted.

Understanding antibiotic resistance mechanisms informs strategies for treatment of *B. pseudomallei* infections. Such information can also be exploited for diagnosis of resistant bacteria.

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Update on treatment of melioidosis

Dr. Wirongrong Chierakul is working as a senior clinical researcher and lecturer at the Faculty of Tropical Medicine, Mahidol University. She graduated from the Thai Board of Internal Medicine from Mahidol University and has a Ph.D. from Open University, UK. She is the Deputy Head of the Department of Clinical Tropical Medicine, and also the Director of Master of Clinical Tropical Medicine Programme.

Melioidosis, a Gram negative bacterial disease, is endemic in Southeast Asia and Northern Australia. Clinical manifestations of the disease vary from mild localized disease to severe fatal septicaemia. The mortality rate remains high and may reach 30% in some areas. Treatment is divided into two phases; acute intensive phase and oral eradication phase. Acute intensive phase starts with intravenous antibiotics. Ceftazidime, one of the third generation cephalosporins, remains the gold standard treatment, especially in Thailand. Meropenem is used in patients with severe septicaemia in Australia. Intravenous injection continues for at least 2 weeks or until clear clinical responses occur, such as defervescence, gaining appetite, improved general well-being, etc. Neurological and rheumatological involvements always need longer intensive treatment, thus combination therapy in these groups are controversial. Trimethoprim-sulphamethoxazole for 12-20 weeks remains the first line treatment for oral eradication phase. Patients who are hypersensitive to sulfa should use amoxicillin-clavulanate with at least 3 times daily dosing interval. The regimen is inferior to the first line treatment.

Update on *B. pseudomallei* soil surveillance

Ivo Steinmetz is Full Professor for Hygiene and Microbiology at the Medical University of Graz, Austria and Chair of the Institute of Hygiene, Microbiology and Environmental Medicine. After gaining his MD degree from the University of Mainz and periods of postdoctoral research he took the Diploma Course in Tropical Medicine and Hygiene in London, where he became interested in melioidosis. Current research interests focus on innate anti-bacterial immune mechanisms and on virulence traits and environmental aspects of *Burkholderia pseudomallei* in various parts of Asia and Africa.

B. pseudomallei infection is acquired from the environment through inoculation, aerosols or ingestion. Environmental suitability for *B. pseudomallei* has been predicted throughout many tropical regions from which environmental detection of the pathogen has never been reported. An increasing number of melioidosis case reports from parts of Asia, Africa and the Americas suggest a worldwide, but grossly underreported distribution of this pathogen. Most studies on the environmental prevalence of *B. pseudomallei* in different parts of the world have relied on culture-based methods. However, current culture methods are very laborious, especially for a large-scale screening and seem to have limited and variable sensitivity in soil samples of various origin. Major parts of potential endemic areas have never been examined for environmental *B. pseudomallei*. This presentation will address methodological aspects of *B. pseudomallei* environmental surveillance and will discuss the usefulness of quantitative multi-target based molecular approaches. Moreover, abiotic and biotic parameters which might determine the environmental presence of *B. pseudomallei* in different habitats will be addressed. Results of a collaborative study with the Sri Lankan team, led by Dr. Enoka Corea, using quantitative direct molecular screening for *B. pseudomallei* in soil samples from Sri Lanka, together with attempts to culture the pathogen from those samples will be presented. Understanding of the ecological factors that determine the environmental dissemination and persistence of *B. pseudomallei* will be important for undertaking any preventive measures. Standardization of sampling methods, sample analysis and biobanking will be fundamental for understanding the global epidemiology of *B. pseudomallei* and melioidosis.

Soil Epidemiology in South West India

Tushar Shaw has completed his Masters in Medical Microbiology from Kasturba Medical College Manipal and is currently working as a Senior Research Fellow at Dept. of Microbiology Kasturba Medical College Manipal. He has undergone training in various aspects of research methodology and molecular epidemiology. He is currently pursuing his PhD on studying the influence of environmental factors on the presence of *B. pseudomallei* in the soil.

Melioidosis, caused by the soil saprophyte *Burkholderia pseudomallei,* is a potentially fatal tropical infection. The disease was under-diagnosed in India, as in many other South Asian countries; however, with increasing knowledge in recent times, there is an upscale in the number of diagnosed cases from different parts of the country. India might have the highest burden of the disease with an annual mortality of nearly 32,000, as predicted recently, emphasizing that the disease should be considered an important public health issue in India.

Environmental surveillance detects the presence of the bacterium in specific geographical areas where humans are more prone to infection. It may help develop a pragmatic framework to develop a wide network for monitoring and evaluating case finding interventions, as well as to implement suitable policies for early detection, management and prevention of the disease.

Majority of the studies for environmental surveillance rely on culture based techniques which are laborious and have limited sensitivity. Hence a sensitive and prompt detection technique is a requisite to study the environmental influence on the persistence of the pathogen in the nature with various ecological factors in abundance. The South West coast of India is the home for tropical and subtropical moist broadleaf forest, high rainfall during the monsoon and rich organic soil, highly suitable for the persistence of the bacterium in the environment. Molecular approaches such as quantitative PCR techniques have shown promising results in understanding the distribution and burden of *B. pseudomallei* in the soil. The survey of *B. pseudomallei* in the soil of South West India is an approach to provide an ideal frame for environmental exploration of the bacterium in other parts of the country.

'Omics' of *B. pseudomallei*

Prof. Tim Inglis is a Professor of Pathology at the School of Pathology and Laboratory Medicine, University of Western Australia and Consultant Medical Microbiologist, Department of Microbiology, PathWest Laboratory Medicine. Perth, Western Australia where his team uses systems biology methods to study melioidosis. Recently completed projects include a US government-funded project on *Burkholderia pseudomallei* in which Prof Inglis was the lead investigator for the biogeographic attribution subproject.

Burkholderia pseudomallei, the cause of melioidosis, has been subjected to intense scrutiny across a range of 'Omics' systems from genome, to transcriptome, proteome and metabolomics analysis. The combination of two or more of these methods into a multi-layered approach is known as integrated systems biology. *B. pseudomallei* has been subject to extensive whole genome sequencing, its core genome extensively annotated and corresponding transcriptome explored. The rich pool of openly accessible *B. pseudomallei* genome data has clarified the molecular epidemiology, phylogenetic proximity of near-neighbour species and organisation of key operons. Proteomic analysis with MALDI-TOF is now in use to assist laboratory identification of *B. pseudomallei* isolates from clinical samples, and has been used as an early warning in an outbreak investigation. The large genome of *B. pseudomallei* corresponds to an extensive repertoire of metabolic functions that enable bacterial adaptation to and survival in a wide range of environmental niches. The volatile organic compounds generated by active *B. pseudomallei* include sulphur compounds that explain the earthy, truffle-like odour of mature laboratory cultures. Single cell cytomic analysis of viable *B. pseudomallei* is at an early stage, but promises to set more mature genomic and other molecular analyses in a cellular context. The combination of these emerging analytical methods is expected to improve our understanding of disease pathogenesis, and open up new route to diagnosis, treatment and prevention.

P01-Targeting intracellular *Burkholderia* by antibody and antibiotic combination therapies

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Introduction

The ability of *Burkholderia pseudomallei* to survive intracellularly, avoiding traditional antibiotic therapy, highlights the importance of investigating novel anti-microbial therapies. This project aims to target *B. pseudomallei* intracellularly with an antibody-antibiotic conjugate. Antibody-antibiotic conjugates as therapies have the ability to target bacteria directly, localising the delivery and functionality of antibiotics. The antibiotic is only functional once cleaved from the antibody at the target site of infection; this has the potential to reduce current antibiotic treatment doses and duration of therapy.

Methods

We are currently developing and utilising *in vitro* macrophage infection assays with *Burkholderia thailandensis* and *B. pseudomallei* to investigate antibody opsonisation and the effect of antibodies on bacterial fate. Antibiotics will be assessed in these assays in combination with free antibodies, this will be compared with an antibody-antibiotic conjugate which is being developed to deliver antibiotic intracellularly to the site of infection. We are using imaging flow cytometry and confocal microscopy to visualise and quantify bacterial infection within macrophages with *Burkholderia* strains expressing green and red fluorescent protein.

Results

Results from macrophage infection assays show that monoclonal antibodies directed against the capsule of *Burkholderia* can significantly increase bacterial uptake by macrophages ($P < 0.0001$), a greater than 1 log increase in colony forming units was observed when bacteria were opsonised with anti-capsule antibody compared to control antibody. Multi-spectral imaging flow cytometry confirmed this result with an increase in intracellular *B. thailandensis* from a control level of 15% up to 40% when opsonised.

Discussion and Conclusion

In conclusion, a *Burkholderia* macrophage infection assay has been created in which bacterial infection can be assessed *in vitro*. Monoclonal antibodies specific against *B. pseudomallei* capsule polysaccharide have demonstrated significant opsonisation ability *in vitro*. This data in combination with *in vivo* protection studies will be used to down select antibodies for conjugation to antibiotics. This represents the first steps towards developing a novel treatment for *Burkholderia* infection.

P02-Platelets aid in host defense during melioidosis

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Introduction

Melioidosis, caused by the Gram-negative bacterium *Burkholderia pseudomallei,* is an important cause of community-acquired pneumonia and sepsis in Southeast Asia with a mortality of up to 40%. Recently, it has been shown that thrombocytopenia is associated with mortality. However, the role of platelets in pathogenesis of melioidosis is unknown. The objective of this study was to assess the role of platelets in the host response during *B. pseudomallei* infection.

Methods

Mice treated with a low or high dose of platelet-depleting antibody (depletion to <5% or <1% of normal, respectively) or IgG control were inoculated intranasally with *B. pseudomallei* and sacrificed at 24, 48 and 72 hours. *B. pseudomallei* growth was studied in mice lacking either platelet Toll-like receptor (TLR) signaling (Platelet factor 4-Cre- Myd88-Lox mice) or Glycoprotein-Ibα signalling (GPIb/IL4R mice) and in mice with impaired neutrophil extracellular trap (NET) formation (PAD4-/- mice). *Ex vivo* human neutrophils were inoculated with *B. pseudomallei* and assessed for internalization by electron microscopy with or without platelet supplementation.

Results

During experimental melioidosis, mice developed thrombocytopenia. Platelet depletion increased mortality and bacterial growth in both lung and liver. Platelet depletion also increased chemo and cytokine responses, but reduced pulmonary neutrophil influx. Mice with deficient NET formation had increased bacterial growth in the blood. However, in platelet-depleted mice NET formation was not impaired. Platelet TLR signaling did not influence bacterial growth but mice lacking platelet GPIbα showed increased bacterial growth in the lung with decreased platelet counts. Platelet depletion also resulted in bleeding in the lung, not seen in uninfected mice. In human neutrophils, platelets increased *B. pseudomallei* internalization and altered phagosome morphology.

Discussion and Conclusion

During experimental melioidosis, platelets play a protective role in host defense and prevention of bleeding.

P03-Prevalence of environmental *Burkholderia pseudomallei* in Sri Lanka

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Introduction

During the last decade, 78% of all recorded melioidosis cases throughout Sri Lanka were detected in the years between 2014 and 2016. A growing awareness of the disease and improvements in diagnosis have presumably led to this remarkable increase. In contrast, our knowledge on the environmental distribution of *Burkholderia pseudomallei* and the preferred habitats of the pathogen in Sri Lanka is still limited. Therefore, this study aimed at identifying the environmental prevalence of *B. pseudomallei* in various parts of Sri Lanka using a previously established quantitative molecular screening approach.

Methods

In February 2016, we performed a soil surveillance study including four sites known for their close proximity to diagnosed cases of melioidosis in the North Western Province, the Central Province and the Eastern Province. In December 2016, we extended our surveillance screening to locations throughout the whole country. We included sites of different soil types and cultivated land, e.g. grassland, dry rice fields, wet rice fields, garden soil and tea plantations in North, South, West and Central Sri Lanka. Overall, 249 subsurface soil samples from about 10cm and samples from about 30cm depth were collected, transferred to Austria and subjected to a molecular screening using a quantitative real time PCR with the *B. pseudomallei*-specific TTSS1 gene as the target.

Results

More than 68% of all samples were *B. pseudomallei*-positive, with individual samples displaying a burden of up to 10^6 genome equivalents per gram of soil. Positivity varied significantly between and within the different sites. Attempts to isolate *B. pseudomallei* in culture in order to analyze the phylogenetic relationship of putative strains compared to clinical strains are ongoing.

Discussion and Conclusions

Our results indicate that *B. pseudomallei* is widely distributed throughout the country. In future, we plan further, extended environmental sampling to unravel the national prevalence of the pathogen in soil and other potential environmental habitats. A thorough knowledge about the ecological factors influencing the lasting establishment of *B. pseudomallei* will be important for any future preventive measures.

P04-MLST analysis of *Burkholderia pseudomallei* isolates from Sri Lanka

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Introduction

Burkholderia pseudomallei is a Gram-negative bacterium found in soil and water in tropical and subtropical regions worldwide. It causes melioidosis, a severe disease with a broad spectrum of clinical presentations that may include joint pain, cough, skin infections, lung nodules and pneumonia. It is hyperendemic to Southeast Asia and northern Australia. The endemic area includes Sri Lanka. A project to screen undifferentiated fever cases to identify potential melioidosis cases resulted in a dramatic rise in the number of culture confirmed cases with over 50 cases being reported each year in recent years. Although the majority of strains are YLF, around 15% of strains belong to the Australian BTFC cluster and a dominant exclusive Australian ST was found to be present in Sri Lanka.

Methods

B. pseudomallei clinical isolates from patients in Sri Lanka were characterized using a molecular genotyping method, multilocus sequence typing (MLST). Sequence types (ST) and clinical data were submitted to the international *B. pseudomallei* database (http://pubmlst.org/bpseudomallei/). The database was used to aid in understanding ST abundance.

Results

A total of 108 strains, clinical isolates from 2006 to 2015, were genotyped. A further 70 strains are in the process of being submitted to the public database. The preliminary results of analysis of twelve samples have shown a high genotypic variation. Out of these, five strains were found to be novel. Of the remaining seven, two were of Indian and Thai origin. As of August 2017, Sri Lanka has the largest representation of all the South Asian countries in the international *B. pseudomallei* database with a total of 109 isolates accounting for around 2.27% of the entire database.

Discussion and Conclusion

A higher resolution genotyping approach (i.e. whole genome sequencing) is needed for a comprehensive comparison between these strains and to understand the presence of the Australian BTFC cluster plus the dominant exclusive Australian ST in Sri Lanka.

P05-Determination of optimum dilutions of extracted antigens from *Burkholderia pseudomallei* strains of Sri Lankan origin for the diagnosis of melioidosis using the indirect hemagglutination assay (IHA)

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Introduction

Burkholderia pseudomallei is the causative agent of the severe community-acquired infectious disease called melioidosis. This bacterium is a Gram-negative saprophytic bacillus that resides primarily in soil and water. The indirect hemagglutination assay (IHA) has found widespread use for sero-surveillance in areas endemic for this disease and has a limited use in the diagnosis of acute melioidosis. However, standardized reagents for the IHA are not available commercially and the test has to be established in-house. The purpose of this study was to extract antigens from different *B. pseudomallei* strains of Sri Lankan origin, determine the optimal dilution of each strain in the IHA test and create suitable combinations of strains for use in our laboratory.

Methods

Ten strains of *B. pseudomallei* were picked from the Sri Lanka *B. pseudomallei* culture collection and subcultured on blood agar plates. Each strain was then inoculated into flasks containing protein free media and incubated at 37° C for two weeks. Each culture was subcultured to check for purity. To obtain the antigen the broth cultures were autoclaved at 15 lbs. pressure for 15 minutes followed by ultra-centrifugation at 20,000 rpm for 30 minutes. The supernatant containing the purified antigen was transferred to fresh tubes and phenol added.

Sheep erythrocytes were sensitized with serial dilutions of the extracted *B. pseudomallei* antigens and tested against a standard positive control serum. Antigen from the strain BPs7 at a dilution of 1/80 was considered the standard. Once optimal dilutions of each antigen were determined, combinations of antigens were prepared by creating pools of 3 antigens each and further tested. The most successful combinations were run along with the standard for routine clinical testing and the results compared.

Results

Antigens BPS 1, BPS 11, BPS 12 and BPS 13 gave satisfactory results relative to the standard. The combination of antigens BPS 11, 12 and 7 proved to be the most effective combination.

Discussion and Conclusions

Antigens extracted from *B. pseudomallei* strains show difference degrees of effectiveness in the IHA test. Each laboratory should perform quality control of extracted antigens before incorporating them into routine laboratory testing.

P06-Epidemiology, presentation and outcome of patients with melioidosis in a tertiary care center

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Introduction

Melioidosis, caused by the Gram negative bacterium *Burkholderia pseudomallei*, is transmitted to humans through direct contact with contaminated soil and water. This research study aimed to describe the epidemiology, presentations and outcome of patients with melioidosis.

Methods

Surveillance was carried out from 1st December 2014 to 30th June 2017 and included all culture and antibody positive patients. In the absence of a positive culture, patients with antibody levels >40 were included if clinical evidence of melioidosis was present. Data were collected from the patients and clinical notes.

Results

Melioidosis was diagnosed in 47 patients during this period of whom 36 (77%) were males. The 41-60 year age group were the most affected (n=25, 53%) with only 3 paediatric (<10 years) and 13 elderly (> 60 years) patients. All the patients were from the Southern Province of Sri Lanka, more than 50% giving a history of constant soil exposure and 6 had been affected by floods. Eighteen (39%) patients were diagnosed by isolation of the bacterium from blood, sputum, pus or urine, with or without a positive antibody test. Twenty nine patients were identified only by a positive antibody test with a compatible clinical presentation. Blood culture was positive in 15 patients. A total of 32 (68%) patients had comorbidities of whom 20 (42%) were diabetic. One was pregnant and one had post-partum pneumonia. Lungs were commonly affected with12 (26%) presenting with severe pneumonia and 10 (21%) with lung abscess. There were 4 patients with liver abscess, 4 with septic arthritis and 2 cases of endocarditis. Other presentations included deep seated abscesses, cellulitis and urinary tract infection. A focus could not be identified in 5 patients. Case fatality rate was 11%. Thirty three patients completed the eradication phase with no relapses while 6 are still undergoing treatment.

Discussion and Conclusions

In this study population, melioidosis had a male predominance and affected the middle aged and elderly with comorbidities including diabetes. A high index of suspicion led to meticulous investigation and early diagnosis, especially among patients with a history of high risk exposure to soil and water.

P07-A serosurvey for anti-*Burkholderia pseudomallei* antibodies in peridomestic rats caught in rural farming and urban sites in Sri Lanka

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Introduction

The Sri Lankan National Melioidosis Surveillance Program has recently revealed nationwide distribution of invasive melioidosis that has placed Sri Lanka as one of the most endemic countries for melioidosis in South Asia. The causative bacterium is commonly acquired from the environment. There are no reported studies from Sri Lanka to identify exposure to *B. pseudomallei* in peridomestic animals. Thus, a serosurvey was conducted to determine the seroprevalence of anti-*B. pseudomallei* antibodies among peridomestic rats infesting an urban setting (Kandy) and a rural farming community (Girandurukotte) in Sri Lanka.

Methods

Rat (*Rattus rattus)* sera, collected under a research project of rodent-borne zoonotic diseases, were obtained from the Department of Microbiology, Faculty of Medicine, University of Peradeniya, Sri Lanka. Ninety two and 157 rat sera from Girandurukotte (GK), Badulla District and Kandy (KY), Kandy District respectively, were analyzed. Presence of anti-*B. pseudomallei* antibodies was established by an ELISA based on lipopolysaccharide (LPS) extracted from *B. pseudomallei* Bp82. The results were validated by Western Blot (WB) analysis with various *B. pseudomallei* LPS types and cell lysate. Cut-off values were determined statistically based on ELISA results.

Results

Of rat sera, 41 (45%) from GK and 47 from KY (30%) were seropositive by ELISA and confirmed by WB. In the WB assay, clear bands were shown to *B. pseudomallei* O-antigen type A and its cell lysate.

Discussion and Conclusions

The current study has shown that rats captured from both rural and urban sites have been exposed to *B. pseudomallei* or *B. thailandensis* since the *B. pseudomallei* O-antigen type A is known to be found in *B. thailandensis*, a non-pathogenic soil bacterium. However, *B. thailandensis* has not been reported in Sri Lanka. Thus, we recommend further in-depth analysis to confirm current findings.

P08-Melioidosis among returning workers in Bangladesh: imported or endemic?

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Introduction

In spite of significant sero-epidemiological surveillance reports and identification of *Burkholderia pseudomallei* from soil specimens, till date, only a few cases of melioidosis, mostly occurring in the north-east region of the country, have been reported in/from Bangladesh. We report four cases of melioidosis occurring among returning workers from endemic countries. Their home districts in Bangladesh were also reported as endemic for melioidosis. Our objective is to create awareness among physicians on the endemicity of melioidosis, both within and outside Bangladesh.

Methods

Patients' socio-demographic, clinical and laboratory data and treatment outcomes were recorded in case record forms.

Results

A 31-year-old plumber, newly detected as diabetic, returned to Bangladesh from Brunei with a diagnosis of septicaemic melioidosis with multiple hepatic and splenic abscesses and portal vein thrombosis. He initially presented with fever and chills. His initial treatment consisted of ceftazidime and amoxicillin-clavulanic acid combination was prescribed in continuation phase. Two months previously he had visited his home district Tangail, endemic for melioidosis in Bangladesh.

A 28-year-old diabetic man from Tangail, presented with fever and back pain. He was diagnosed as having spondylo-discitis and paravertebral abscess due to *B. pseudomallei* infection. Brucellosis and tuberculosis were excluded. He was treated with ceftazidime followed by co-trimoxazole and doxycycline. He used to work as carpenter in Saudi Arabia and returned home three months previously.

A 43-year-old newly detected diabetic carpenter, returning from Brunei, presented with three-month history of recurrent fever, cough, sputum production and weight loss. He had been treated for pneumonia in Brunei and anti-tuberculosis medication was prescribed empirically at Brahmanbaria, his native district in Bangladesh, without much benefit. Subsequently, sputum culture grew *B. pseudomallei* and he recovered with ceftazidime followed by doxycycline and co-trimoxazole.

A 37-year-old diabetic construction worker presented with non-resolving pneumonia for two months. He came back to Tangail three months ago from Singapore. Tuberculosis was excluded and blood culture grew B. pseudomallei. He was treated with meropenem followed by amoxicillin-clavulanic acid combination with complete resolution.

Discussion and Conclusions

Melioidosis is endemic in Bangladesh. Any patient with fever should raise suspicion of melioidosis, especially if diabetic and from endemic districts of Bangladesh or returning from endemic countries.

P09-Melioidosis in northern Sri Lanka: filling the gap in the map

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Introduction

National surveillance has established melioidosis as endemic throughout Sri Lanka but data is lacking from the Northern Province. Only one culture-positive case had been reported up to 2013, probably due to lack of clinical microbiology services and misidentification of *Burkholderia pseudomallei* as *Pseudomonas* species due to lack of technical familiarity. This study describes melioidosis in northern Sri Lanka between April 2014 and April 2017.

Methods

Clinical microbiology services at Teaching Hospital, Jaffna were upgraded in 2014. *B. pseudomallei* culture work-up procedure was introduced with reference laboratory support. Technicians were trained using control cultures. Physician awareness was increased. Sample collection for culture and antibody detection from suspected patients was encouraged. Probable isolates were confirmed by real time PCR assays for *Lpx*O and YLF/BTFC targets and serum was sent for antibody testing by the indirect haemagglutination assay.

Results

Six culture-positive cases were detected in 2016, three males and three females, all above 40 years of age. Clinical presentations included sepsis, pneumonia, septic arthritis and abscess. Four patients died (case fatality rate 67%), one recovered and one was lost to follow-up. The causative organism was isolated from two knee joint (KJ) aspirates and five blood cultures (both blood and KJ aspirate culture were positive in one patient). Five patients had significant risk factors for exposure such as rice farming (n=2), flooding (n=2) and construction work (n=1). Three were diabetic and other comorbid conditions (alcoholism, malignancy) were present. Four isolates belonged to the Southeast Asian biogeographic YLF variant and two belonged to the Australian BTFC variant. Antibody titres tested in three of the culture-positive patients were ≥2560. Serum antibody titres were tested in 99 patients and 19 (19.2%) had levels ≥1:40. Three (who remained culture-negative but had antibody titres of 320, >10240 and >10240 respectively) were treated for probable melioidosis and all recovered.

Discussion and Conclusions

These findings address the dearth of information on melioidosis from northern Sri Lanka. Many cases probably remain undetected. Clinical and laboratory diagnosis should be continuously reinforced for improving case detection as early diagnosis has led to reduced mortality elsewhere in the country.

P10-Detection of *B. pseudomallei* DNA among febrile patients presenting to tertiary hospitals in north eastern Malaysia

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Introduction

Melioidosis, caused by *Burkholderia pseudomallei*, is an important infection, endemic to many tropical regions including South East Asia and northern Australia. In Malaysia, it is estimated that 19% of melioidosis patients died within the first 48 hours of admission, due to septic shock. Early detection plays an essential role in patients prognosis but clinical diagnosis of melioidosis is difficult, as other tropical infections, such as leptospirosis, dengue and scrub typhus also portray similar disease manifestations. A better detection method is therefore urgently needed to allow timely diagnosis and accurate treatment for melioidosis. Our study aimed to evaluate the performance of qPCR for the detection of *B. pseudomallei* among febrile patients who presented to healthcare facilities in north eastern Malaysia.

Methods

A total of 108 blood samples from febrile patients were collected between April 2016 and May 2017 from Hospital Sains, Malaysia, Hospital Raja Perempuan Zainab II and Kota Bharu Public Health Laboratory, Malaysia. Genomic DNA were extracted and analysed using real time qPCR to detect the presence of *B. pseudomallei*. Subsequent clinical data, including related microbiological investigation results of patients with positive assay were retrieved to confirm the molecular findings.

Results

Three samples (2.8%, $n = 3/108$) were found to have detectable *B. pseudomallei* DNA. Parallel laboratory investigations revealed that these 3 patients had positive blood cultures. *B. pseudomallei* were isolated in two samples. Meanwhile, the other patient's blood culture grew multiple organisms; *Klebsiella ozanae* and *K. oxytoca*. Upon retrieval of extensive clinical data, the qPCR-positive, culture-negative patient was reported to have liver abscesses and underlying diabetes mellitus type II. This scenario is possible as conventional culture methods have limited sensitivity (~60%). Therefore, multiple sampling and radiological investigation are recommended in patients with suspected melioidosis. We also found that two of the melioidosis patients also had simultaneous leptospirosis, based on the concurrent leptospiral investigations.

Discussion and Conclusions

This study provides preliminary data in favour of utilisation of qPCR as a sensitive screening tool for melioidosis diagnosis in laboratories of tertiary hospitals in Malaysia, and other endemic regions. Moreover, it also highlights the possibilities of misdiagnosis of melioidosis /leptospirosis, in the event when only a single laboratory test is available or requested.

P11-The epidemiology and clinical manifestation of neurological melioidosis: A series of 19 cases from Southwest India over 10 years

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Introduction

Melioidosis, a syndrome with protean clinical manifestations, is caused by a Gram negative soil saprophyte, *Burkholderia pseudomallei*. Among its diverse clinical presentations, pulmonary, cutaneous, osseous, deep-organ, and septicemic foci are well-known. Neurological manifestations are however, less frequently observed and the microbiological evidence of such is even more rare. This study was undertaken to obtain newer insights into the clinical spectrum of neurological melioidosis, including its presentation, microbiological and radiological diagnosis and associated risk factors.

Methods

Neurological manifestations in patients diagnosed with culture confirmed melioidosis were documented during the period of January 2006 to December 2016. Basic demographics and risk factors associated with neurological complications were determined.

Results

Among 200 patients with melioidosis, 19 (9.5%) presented with neurological manifestations. The mean age was 46.8 ± 13.9 years (range 7-67 years). The majority of the patients were male (16/19; 84.2%). Most presented during the monsoon (14/19 73.6%). Common clinical presentations observed were fever (16; 84.2%), headache (63.5%), paresthesia (47.3%) and lower limb paresis (36.8%). Magnetic resonance imaging (MRI) showed that brain abscess was the most common finding. Diabetes mellitus was the most common risk factor (68.4%). Blood culture had more diagnostic value than CSF culture in patients with neurological melioidosis. Overall mortality was 31.5% (6/19).

Discussion and Conclusions

Neurological signs and symptoms have not been classically associated with melioidosis. Treating physicians should consult the clinical microbiologist whenever they have any suspicion of such cases and the microbiology laboratory should be well-equipped to diagnose melioidosis from CSF and/or blood samples.

P12-*Burkholderia pseudomallei*: case series from the Provincial General Hospital, Kurunegala

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Introduction

Burkholderia pseudomallei, a Gram negative, bipolar-staining, oxidase-positive, non-fermenting bacterium is the aetiological agent of the clinical condition named melioidosis. It is a soil saprophyte, commonly causing infections in susceptible populations in agricultural areas. Infection is acquired by inoculation via breaches in the skin, inhalation of soil or ingestion of water contaminated by the bacterium. Melioidosis is common in tropical and subtropical regions of South East Asia and is an emerging pathogen. Sri Lanka also shares similar climatic conditions which result in vulnerability to the disease. The Kurunegala District in Sri Lanka is situated in the North Western Province which is an agricultural area with mainly paddy farming. Susceptible population includes farmers, diabetics and people with chronic kidney disease.

Case series

The Microbiology Laboratory of the Provincial General Hospital (PGH), Kurunegala isolated *B. pseudomallei* identified by latex agglutination and PCR at the Department of Microbiology, Faculty of Medicine, University of Colombo from 36 patients in a 15 month period from January 1st 2016. Of the 36 isolates, 21 were from blood culture in patients with septicaemia, 3 of whom died. Eleven isolates were from abscesses, one from a patient with septic arthritis and 3 from patients with bacteremia with no identified focus. Two patients had a positive culture from sputum. Seventeen patients of the 36 patients had diabetes mellitus.

Discussion and Conclusion

Increased awareness among clinicians and improved diagnostic capability have contributed towards the increased diagnosis of melioidosis in Kurunegala hospital. The majority of patients had a favourable clinical response. Active participation and collaboration with all stake holders will be continued and expanded so that patients with melioidosis presenting to this hospital would be facilitated by early diagnosis and initiation of appropriate treatment, thus reducing both morbidity and mortality associated with this disease.

P13-Evaluation of treatment outcomes of intra-venous ceftazidime as the intensive phase anti-microbial in melioidosis – a case series

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Introduction

Melioidosis is an infection caused by the Gram negative bacterium, *Burkholderia pseudomallei.* It is associated with high morbidity and mortality even after treatment with potent antibiotics. We present four cases of culture positive melioidosis, treated with intravenous ceftazidime as the antimicrobial agent in the intensive phase. Our main objectives were to analyze morbidity, mortality and time taken for clinical remission following intensive phase treatment with intravenous ceftazidime.

Methods

This is a retrospective study carried out over a period of 6 months from January 2017 at the Teaching Hospital Batticaloa, Sri Lanka. All culture proven cases of melioidosis admitted to medical and surgical wards were included. Intravenous ceftazidime for 28 days, with or without co-trimoxazole, was given to all patients during the intensive phase. Absence of fever and a reduction of the CRP by 50% of its highest value were taken as indicative of clinical remission.

Results

There were four culture positive patients of whom three had positive blood cultures and one had a positive sputum culture. They showed nonspecific clinical presentations such as skin abscesses, splenic abscesses, lung abscesses and septic arthritis. All patients had near total recovery after intensive phase of chemotherapy for 28 days and there were no deaths compared with the high mortality rate in disease endemic areas of the world. This could be due to early clinical detection and appropriate and long course of antibiotics. Mean fever clearance time was 8.33 days. Mean time taken for highest CRP value to reduce by 50% was 6.66 days. Early clinical remission in patient with septic arthritis may be due to arthrotomy and washout.

Conclusion

The satisfactory outcome of these cases highlights the importance of early diagnosis with a high index of suspicion, correct use of antibiotics and improved microbiology services. However, adequate sample size is needed to determine the clinical significance of these findings.

P14-Osteoarticular melioidosis: A bone to pick about an emerging but neglected tropical disease

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Introduction

Melioidosis, caused by the soil inhabitant *Burkholderia pseudomallei*, often presents with osteoarticular manifestations in endemic settings, where the mode of infection is through inoculation. Bacteraemic forms of the disease, which is associated with high case fatality rates, are often due to the systemic dissemination of bacteria from osteoarticular infections. With increasing evidence suggesting the endemicity of melioidosis in India, the present study was undertaken to elucidate the clinical, microbiological and epidemiological characteristics of patients diagnosed with osteoarticular forms of melioidosis.

Methods

Data obtained from 200 patients diagnosed with melioidosis (culture-confirmed) from January 2006 to December 2016 at a tertiary care teaching hospital in South-West India was used in the present study. Baseline demographic and clinical characteristics of patients with osteoarticular form of melioidosis among the study cohort were further analyzed.

Results

Osteoarticular melioidosis accounted for 17.5% (35/200) of all cases of melioidosis in the given period, comprising of 29 (82.8%) males and 6 (17.1%) females. Mean age of the study population was 47.7 ± 13.6 (Range 7-67). Twenty-four cases (68.5%) presented with mono-articular septic arthritis, while 11 (31.4%) had osteomyelitis. The most commonly affected site was the knee joint (25, 71.4%), followed by vertebrae (3; 1 thoracic and 2 lumbar) and ankle joint. Diabetes mellitus was the most common co-morbid condition (29, 82.9%), followed by chronic alcoholism (9, 25.7%) and chronic kidney disease (6, 17.1%). Bacteremia was documented in 15 (42.8%) cases. Case fatality was 8.5% (n=3).

Discussion and Conclusion

Early diagnosis and befitting management of osteoarticular melioidosis can prevent systemic dissemination of the bacteria and thus reduce the associated case-fatality rates. Microbiological culture results play an indispensable role in diagnosis of osteoarticular melioidosis.

P15-Melioidosis case cluster following heavy inter-monsoonal rains in the Eastern Province of Sri Lanka

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Introduction

Melioidosis is an emerging tropical infection caused by the soil bacterium *Burkholderia pseudomallei*. Sri Lanka has recently been identified as endemic for the disease. The study describes a cluster of cases of acute bacteraemic melioidosis following an extreme weather event. This study describes the clinical, epidemiological and bacteriological characteristics of a cluster of bacteraemic melioidosis cases.

Methods

Cultures from patients were referred to the reference laboratory and confirmed by real time polymerase chain reaction (RT-PCR). Clinical histories were recorded. Multi locus sequence typing was performed for genotyping. Rainfall data in Batticaloa for October 2015 was obtained from the Department of Meteorology of Sri Lanka.

Results

Ten culture positive, adult cases of melioidosis were identified in the Batticaloa District of the Eastern Province of Sri Lanka between November and December 2015, following heavy rainfall in October. All had direct or indirect exposure to flood water. Only two were farmers. Six had diabetes mellitus. All ten cases presented with fever and bacteraemia. Focus of infection was identified in 8 patients (pneumonia - 3, sinusitis - 2, joint infections - 2 and pyelonephritis - 1). Four patients (3 pneumonia and one with pyelonephritis) died, giving a case fatality rate of 40%, which is significantly higher than the case fatality rate of 23% recorded in sporadic cases. Although two had co-morbidities, two were healthy young females who presented with pneumonia, septic arthritis (indicating dissemination) and septicaemia and had a severe acute course. Four isolates were YLF while 6 isolates belong to the BTFC gene cluster which is uncommon in Asia, with four isolates being of a single multilocus sequence type (St594).

Discussion and Conclusion

A single ST (ST594) was responsible for a significant proportion (40%) of cases. The reason for this is unclear. Inadequate community-based detection and control of diabetes was the major underlying factor. Late presentation, poor control of diabetes and late recognition contributed to the high mortality. Primary prevention of melioidosis is difficult because of the saprophytic nature of the organism and the inevitability of flooding during the rainy season. Secondary prevention, by early suspicion and culture confirmation, requires close coordination between clinicians and microbiologists.

P16-Kidney manifestation of melioidosis: a study from a tertiary care hospital in South India

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Introduction

Melioidosis is caused by the facultative intracellular Gram-negative bacterium, *Burkholderia pseudomallei* clinically ranging from subclinical infection to pneumonia or skin involvement. The disease can involve any organ. Diabetes mellitus and chronic kidney disease (CKD) are risk factors. We describe the renal manifestations of melioidosis in our population and analyze their impact on disease outcome.

Methods

Consecutive culture confirmed melioidosis patients were recruited from January 2006 to December 2016 and analyzed. Chronic kidney disease was defined as evidence of kidney damage or estimated glomerular filtration rate (GFR) less than 60 mL/min/1.73m^2 for three months or more. Acute kidney injury (AKI) was defined as an increase in serum creatinine by 0.3mg/dL or more within 48 hours or increase in serum creatinine to 1.5 times baseline or more within the last 7 days or urine output less than 0.5 mL/kg/h for 6 hours. An estimated GFR 75mL/min/1.73m^2 was considered as normal. Basic demographics of the study population were studied and the associations of the renal dysfunction were analyzed for disease outcome and presentation.

Results

Of 200 individuals with melioidosis 34 (17%) had renal impairment including 21(10.5%) with CKD and AKI in 16 (8%). Mean age was 46.6 ± 15.8 years and 160 (80%) were male. Both CKD and AKI were associated with an increased chance of developing bacteremia ($p=0.001$).Mortality was seen in seven (33.3%) with CKD ($p<0.001$) and in seven (43.7%) with AKI ($p<0.001$). Sepsis with AKI was seen in (28.5%) ($p<0.001$).

Conclusion

Melioidosis causes AKI in 8% and kidney dysfunction predisposes to bacteremia and mortality.

P17-Melioidosis: variety of manifestations and risk factors

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Introduction

Knowing the risk factors and clinical manifestations of melioidosis will help identify and treat patients early leading to better outcomes. This study describes the clinical manifestations and risk factors in patients with culture proven melioidosis identified at the Sri Jayewardenapura General Hospital (SJGH).

Methods

Data of patients who had culture positive melioidosis at SJGH from January to May 2016 were extracted from the WHONET database and relevant clinical details gathered from hospital records.

Results

The first patient was a 60 year old male who presented with intermittent fever for one month and nonspecific abdominal symptoms. He was a known diabetic for 12 years. Abdominal imaging revealed a liver abscess. Pus from the abscess grew *Burkholderia pseudomallei*.

The second patient, a 36 year old male also presented with intermittent fever for one month. He was a known diabetic and an occasional alcoholic. Imaging studies showed multiple liver and splenic abscesses. Blood culture became positive for *B. pseudomallei*.

The third patient, a 55year old trishaw driver who transported compost fertilizers presented with intermittent fever for 1½ months, loss of appetite and left knee joint pain. He had defaulted treatment for diabetes mellitus for 6 years and was a heavy alcoholic. Multiple liver and splenic abscesses and septic arthritis were diagnosed. Blood culture and joint aspirate became positive for *B. pseudomallei*.

The fourth patient, a 28 year old tipper driver from Elpitiya, who transports rubble, presented with fever for 3 weeks, loss of weight and loss of appetite. He had multiple subcutaneous nodules over the arms and legs. This patient was a known asthmatic and on a steroid inhaler. Blood culture was sterile but aspirated fluid from the subcutaneous nodules yielded *B. pseudomallei*.

All but the third patient recovered after appropriate treatment.

Discussion and Conclusions

All four patients had a prolonged history and known risk factors. Possible occupational risk factors were noted in patients 3 and 4. Melioidosis should be suspected in patients presenting with prolonged fever and suppurative lesions and relevant radiological and microbiological investigations carried out to initiate appropriate treatment.

P18-Case Report: Melioidosis – poor compliance resulting in a relapse ?

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Introduction

Melioidosis is an emerging infection in Sri Lanka, acquired by inoculation or inhalation of soil and water containing *Burkholderia pseudomallei*. The disease may be acute, chronic, localized or disseminated.

Case report

A 55 year old male with poorly controlled diabetes mellitus presented with fever for 5 days and left sided abdominal pain in January 2017. Two years previously, just after returning from Malaysia, he developed recurrent episodes of a neck abscess. His CRP was 192 mg/L. Ultrasound abdomen revealed splenomegaly with multiple focal lesions. Blood culture grew *B. pseudomallei* with a positive antibody titre of >1280. He was treated with IV ceftazidime and oral cotrimoxazole for two weeks and discharged on oral cotrimoxazole for ten more weeks.

Five months later he presented with fever, left sided abdominal pain and difficulty in breathing for 5 days and admitted discontinuation of the eradication phase cotrimoxazole after 4 weeks. The white cell count was 21.47 x 10^9/L and CRP was 185 mg/dl. Ultrasound abdomen showed a small subphrenic collection with splenic abscesses. On the CT scan, there were empyema of the left lung, a subphrenic collection and multiple abscesses in the spleen, liver and kidneys. Aspirated pus grew *B. pseudomallei* after 4 attempts and prolonged incubation of the sample. The melioidosis antibody titre was >10240. On admission, he was started with IV ceftazidime, oral doxycycline and cotrimoxazole in high doses. The patient improved clinically and was discharged after counselling on completing the eradication phase of treatment.

Discussion and Conclusion

Recurrent melioidosis may be caused by relapse or reinfection. Inadequate intravenous antibiotics, multifocal infection, bacteraemia, disseminated melioidosis during the primary episode and inadequate duration and poor compliance of eradication therapy are associated with recurrences. The second episode is a probable relapse which was not confirmed due to unavailability of genotyping facilities. Knowledge on the nature of the disease with its propensity to relapse, prompt aspiration of abscesses and repeated attempts at culture were important in confirming the relapse in this case. It is the responsibility of the clinicians to counsel the patient on discharge about the importance of compliance with the eradication treatment to prevent life threatening relapses.

P19-Case Report: A patient with melioidosis presenting with fever, dysuria and hip pain – a diagnostic dilemma

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Introduction

Melioidosis, caused by *Burkholderia pseudomallei*, is endemic in South/Southeast Asia. It is re-emerging in Sri Lanka, probably due to increased awareness among microbiologists and clinicians. However, being a diagnostic dilemma due to its varied presentations, this great mimicker requires a high degree of clinical suspicion for early diagnosis and optimal antimicrobials to reduce morbidity/mortality.

Case report

An elderly diabetic female presented with fever for 1 month, dysuria and left-sided hip pain. WBC and UFR were normal but CRP was high. Urine culture was negative but blood culture became positive, resembling a mixed growth. Upon standing, the culture manifested as a single type of wrinkled colonies with typical features of *B. pseudomallei*. Suggestive antibiogram and non-identification by Vitek® led to reference laboratory identification. Antibodies against *B. pseudomallei* were also high (>10240).

In spite of worsening pain, X-Ray/USS revealed a normal hip joint. Double dose meropenem was started. MRI revealed joint effusion indicating septic arthritis. CT-KUB/CECT-abdomen showed bilateral renal cysts and a peri-nephritic collection. Meropenem dose was lowered after excluding CNS involvement. Oral cotrimoxazole was added. At the time of submission, the patient is on 20 days of induction therapy, is awaiting repeat imaging and is clinically better. Eradication therapy with oral cotrimoxazole/doxycycline for ≥ 3months is planned.

Discussion and Conclusion

B. pseudomallei is a soil-dwelling bacterium. Inhalation or direct inoculation through damaged skin are the routes of transmission. The patient was exposed to her pet dog that brought soil into the house after rolling outside. Septic arthritis is a well-known manifestation of melioidosis. Renal cysts in this patient may represent renal involvement. In this instance, if the blood culture result was disregarded as mixed due to contamination and if the patient's complaint was unheeded due to initially normal investigations, the diagnosis would have been missed. A high index of clinical suspicion is needed for diagnosis and treatment of melioidosis.

P20-Case report: Melioidosis presenting as urinary tract infection

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Introduction

Melioidosis, caused by *Burkholderia pseudomallei* can present with a spectrum of diseases ranging from mild wound infections to severe pneumonia or deep seated abscesses. Genitourinary melioidosis is one of these manifestations.

Case report

A 71 year old non-diabetic male with a history of bladder out-flow obstruction was admitted with acute retention of urine following fever and dysuria for one week. He had worked at the Fisheries Department and had no significant soil exposure history.

On examination, the patient was febrile and had prostatomegaly. The white cell count (WCC) was $22.8x10^9$/L with 80% neutrophils and the CRP was 131 mg/L. Urine full report showed 8-10 pus cells/HPF. Ultrasound scan showed mild prostatic enlargement with no evidence of abscess formation or pyelonephritis.

Urine culture was positive for a non-lactose fermenting, oxidase positive, Gram negative bacillus which was suspected to be *B. pseudomallei*, due to the typical antibiotic sensitivity pattern of co-amoxiclav sensitivity and gentamicin resistance. The identity of the isolate was confirmed by latex agglutination . Melioidosis antibodies were positive with a titre of >10240. Blood culture was negative.

Initial treatment was with intravenous ceftazidime 1g/8 hourly, increased to 2g/8 hourly after confirmation of the diagnosis. Oral doxycycline 100mg/12 hourly and oral cotrimoxazole 960mg/12 hourly were added. Fever decreased with reduction of CRP and WCC. After 17 days there was still a mild elevation of CRP and treatment was changed to meropenem for three weeks, after excluding other septic foci. The patient was discharged on oral cotrimoxazole for eight weeks.

Discussion

Melioidosis is an emerging disease in Sri Lanka which requires a high index of suspicion for diagnosis due to the absence of distinctive clinical features. Though this patient had no predisposing conditions, working in the Fisheries Department could have been a risk factor. Diagnosis was made through accurate identification of the urine culture isolate in spite of urine being an unusual specimen to be culture positive in melioidosis in Sri Lanka. This case strengthens the clinical significance of *B. pseudomallei* as a urinary pathogen.

P21-Case Report: Melioidosis in non-endemic settings: a case with atypical presentation and diagnostic challenges

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Introduction

Burkholderia pseudomallei infection is rare in Europe and usually seen in the context of travel-related infection.

Case report

We report a case of a 43-year-old male from Gujarat, India living in the UK, who developed relapsed *B. pseudomallei* infection on a background of poorly controlled diabetes, alcoholism and malnutrition. He presented in diabetic ketoacidosis and was admitted to intensive care for multi-organ support.

Admission blood cultures flagged positive after 5-days incubation and an oxidase positive, Gram-negative bacillus grew on blood and chocolate agar after 24 hours. The organism was resistant to gentamicin and colistin but sensitive to ceftazidime, meropenem and piperacillin/tazobactam on disc diffusion testing. It was initially poorly identified as *Burkholderia multivorans and Burkholderia cepacia* using MALDI-TOF MS and API 20NE respectively. The organism was sent to the reference laboratory for *B. pseudomallei* PCR which was positive. Urine culture and the dialysis catheter tip subsequently grew *Trichosporon asahii*.

CT head on admission showed no acute abnormality but CT of the chest/ abdomen/pelvis showed bibasal cavitatory lung lesions, splenic infarction, and hypodense lesions in both kidneys. CT head repeated 16 days post-admission showed multiple cerebral abscesses. Trans-thoracic echocardiogram showed no vegetations and sputum was negative for *Mycobacterium tuberculosis* PCR and culture.

Despite initial response to meropenem, he rapidly deteriorated and developed seizures, likely secondary to new intracerebral abscesses. Positive cultures for *T. asahii* suggest secondary superadded fungal infection. Despite treatment with amphotericin B, isavuconazole and meropenem, the patient died.

Discussion and Conclusion

This case serves as a reminder that many cases of melioidosis are relapsed infection rather than recently acquired. Immunosuppression is a significant risk factor for relapse. Mortality is high and optimal treatment regimen for severe melioidosis remains unresolved.

Organism identification in a UK laboratory can prove challenging and some MALDI-TOF MS databases fail to identify *B. pseudomallei* adequately. Therefore, vigilance is required to identify suspicious isolates in order to pursue identification by alternative means.

Melioidosis can occur in patients without a recent history of travel due to relapsing disease. A high level of clinical suspicion is required in order to identify the organism correctly on culture.

P22-Case Report: An unusual cause of cyst infection in adult polycystic kidney disease (ADPKD)

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Introduction

Although many parts of India are endemic for melioidosis, it is still considered an uncommon infection, often unsuspected and under-diagnosed.

Cyst infection is a serious complication of ADPKD accounting for 15% of hospitalization in these patients. Non-specific clinical manifestations, poor yield on urine and blood cultures and the limitations of conventional imaging techniques makes the diagnosis especially challenging.

Case report

A 43 year old diabetic with chronic kidney disease secondary to ADPKD underwent a renal transplant in 2012 and was on maintenance immune-suppressants including low dose steroid. He presented to us in June 2017 with high grade fever and severe right sided flank pain. Initial blood and urine cultures were negative. CT scan of the abdomen revealed hypodensities within cysts of the right native kidney with peri-nephric and peri-ureteric fat stranding involving the transplant kidney as well. FDG PET scan was performed for further confirmation of cyst infection which showed soft tissue thickening along the wall of few large cysts in the right native kidney with significant uptake. The patient underwent right nephrectomy. Intra operatively, infected cysts with necrosis were seen and *Burkholderia pseudomallei* was isolated on tissue culture. On further questioning, the patient revealed that he had traveled to his native village on the Konkan coast a few weeks prior to his illness. He responded well to intravenous ceftazidime and oral cotrimoxazole and was afebrile on discharge.

Discussion

Renal abscesses or pyelonephritis due to melioidosis is a well-documented entity. However, only one such case of cyst infection in a patient with ADPKD has been reported earlier. Thus despite compatible risk factors, melioidosis was not considered in this patient. In fact, the epidemiological history was sought only after identification of the pathogen.

Precise identification of the organism was crucial in this case as *Burkholderia pseudomallei* has an unusual susceptibility pattern and requires prolonged duration of treatment. Currently used empiric treatment is unlikely to be effective.

P23-Case Reports: Experiences with empiricism in melioidosis

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Introduction

We present a case series of five patients who presented to us at a tertiary care centre in Mumbai during the monsoons with history and clinical findings suggestive of melioidosis. We were unable to isolate *B. pseudomallei* from blood or tissues.

Case reports

A 47 year old diabetic, alcoholic farmer presented with high grade fever for one month followed by scrotal pain and ulcer. Therapeutic trial of Trimethoprim-sulfamethoxazole (TMP SMX) was given and he showed a dramatic response.

A 55 year old diabetic housewife living close to paddy fields, presented with persistent fever and left upper quadrant abdominal pain. She had multiple splenic abscesses on CT abdomen and had received anti-tuberculous therapy elsewhere with no response. Symptoms resolved on empiric ceftazidime and TMP SMX.

A 63 year old diabetic paddy farmer developed multiple subcutaneous abscesses on his shins. A swab culture done elsewhere isolated *Pseudomonas spp.* He presented to us in a critical state with a brain stem abscess. We started him on intensive therapy for melioidosis but the patient succumbed within a few days.

A 27 year old presented with a history of increasing left flank pain and fever for one month. An abdominal CT scan revealed multiple left renal abscesses. Empiric therapy for melioidosis resulted in rapid defervescence and resolution of flank pain.

A 62 year old diabetic lady returned from Singapore with fever and back pain for 1 month. MRI spine showed spondylodiscitis and a CT guided biopsy done elsewhere grew *Burkholderia cepacia*. As she had no history of health care contact, she was treated for melioidosis with complete resolution of signs and symptoms.

Discussion and Conclusions

Although India is endemic for melioidosis, it is still considered an unusual exotic infection in most parts of the country. In the above cases, the possibility of melioidosis was carefully assessed based on specific epidemiological and host factors, clinico-radiological findings and the absence of an alternative diagnosis. Every effort was made to isolate the organism, failing which a therapeutic trial was offered. The standard treatment regimen for melioidosis used in our cases is unlikely to be effective for other bacterial infections and hence successful outcome in most cases encouraged us to believe that our clinical suspicion of this infection was not unfounded.

P24-Loop-mediated isothermal amplification assay targeting the virB gene for rapid detection of *Brucella* spp

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Introduction

Diagnosis of brucellosis has always been a dilemma, due to the slow growth rate of *Brucella* spp. in culture and cross reacting antibodies in serological tests. It is therefore crucial to develop more sensitive and specific diagnostic techniques for rapid and accurate diagnosis of brucellosis. This study aimed to develop and evaluate a loop-mediated isothermal amplification (LAMP) assay for rapid detection of *Brucella* spp. in human blood, body fluid and tissue specimens.

Methods

Brucella-specific primers for loop-mediated isothermal amplification (LAMP) were designed using the LAMP primer designing software program http://loopamp.eiken.co.jp/e/lamp/primer.html, targeting the sequence of *Brucella melitensis* 16M virB gene. The developed LAMP was tested for its specificity with *B. melitensis* and 8 non-Brucella isolates. Sensitivity of the developed LAMP was also carried out with known quantity of DNA. Human whole blood, body fluid and tissue samples were collected from suspected and diagnosed cases of brucellosis for the detection of *Brucella* DNA and results were compared with polymerase chain reaction (PCR).

Results

The reaction can be completed in 60 min at 63°C. The sensitivity of LAMP assay was detected to be 100-fold more than the conventional PCR, with a detection limit of 10pg of genomic DNA. No cross-reactivity was observed with 8 non-*Brucella* species. A total of 48 whole blood, 11 vertebral aspirates, 5 tissue and 4 CSF samples were collected from 48 suspected cases of brucellosis. Using the LAMP assay, 4 of 11 vertebral aspirates and 4 of 48 whole blood samples were detected positive for *Brucella* spp., when conventional PCR detected only 2 vertebral aspirates and one whole blood sample. The sensitivity and specificity was 80% and 100% in comparison with culture.

Discussion and Conclusion

LAMP is a suitable rapid molecular technique that could help to reduce the time required to isolate and identify slow growing and fastidious bacteria in low-resource laboratories, mainly in developing countries.

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