UNIVERSITI PUTRA MALAYSIA

ROLE OF Burkholderia pseudomallei EXOTOXIN IN THE PATHOGENESIS OF MELIOIDOSIS USING MICE MODEL

RABIU MUHAMMAD ALIYU

FPV 2016 3

ROLE OF *Burkholderia pseudomallei* EXOTOXIN IN THE PATHOGENESIS OF MELIOIDOSIS USING MICE MODEL

By

RABIU MUHAMMAD ALIYU

Thesis Submitted to the School of Graduate Studies University Putra Malaysia, in Fulfilment of the Requirements for the degree of Doctor of Philosophy

June 2016

COPYRIGHT

DEDICATION

Dedicated to my late father Alhaji Muhammad Aliyu.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in Fulfilment of the Requirements for the degree of Doctor of Philosophy

ROLE OF *Burkholderia pseudomallei* EXOTOXIN IN THE PATHOGENESIS OF MELIOIDOSIS USING MICE MODEL

By

RABIU MUHAMMAD ALIYU

June 2016

Chairman : Noordin Mohamed Mustapha, PhD
Faculty : Veterinary Medicine

Burkholderia pseudomallei is a gram-negative bacterium and a causative agent of melioidosis, a serious often fatal multisystem disease of man and animals. In Malaysia, *B. pseudomallei* have been isolated from different subjects in the states of Pahang, Johor, Kuala Lumpur and Kedah. Strains of *B. pseudomallei* were known to secrete a range of extracellular enzymes (exotoxins) with different lethal activities such as proteolytic, lipolytic, haemolytic activities. However, little study has been conducted on the characteristic of exotoxins from Malaysian isolates. Similarly, there is scarce of information related to the role of the local *B. pseudomallei* isolates and its associated exotoxins in the pathogenesis of melioidosis. This study is aimed to determine the molecular characteristics of *B. pseudomallei* as well as to study the role of its exotoxin in the pathogenesis of melioidosis. Three *B. pseudomallei* isolates designated UPM/2012/1017, UPM/2013/006 and UPM/2013/577 strains used in this study were obtained from cases of animal melioidosis presented to Universiti Veterinary Hospital (UVH), Universiti Putra Malaysia (UPM). The isolates were reconfirmed phenotypically and biochemically followed by confirmation using 16S rRNA sequencing. Sequencing and phylogenetic analysis of the isolates showed that they were closely related to *B. pseudomallei* K96243 and 1026b strains from Thailand as well as *B. pseudomallei* MSHR2543 strain from Australia. *B. pseudomallei* K96243, 1026b and MSHR2543 strains were among highly virulent *B. pseudomallei* strains known globally and most frequently reported from humans, indicating possible zoonotic potentials of these local *B. pseudomallei* strains. This study suggested the existence of fatal animal melioidosis caused by these *B. pseudomallei* strains. Analysis of their extracellular virulence genes (exotoxins) revealed the presence of three genes encoding for phospholipase C and a gene encoding for a protease. Further characterization of the isolated extracellular proteins (exotoxin) by SDS-PAGE analysis indicated the presence of phospholipase C enzyme with a molecular weight of 77 kDa. Following protein characterization, an *in vivo* assay using BALB/c mice was carried out to evaluate the toxigenicity of the extracellular protein isolated. A total of 130 BALB/c mice used in this study were divided into 5

treatment groups: control (Cx) (n =10), live *B. pseudomallei* (Bp) (n =30); live *B. thailandensis* (Bt) (n =30); *B. pseudomallei* exotoxin (E) (n =30) and *B. thailandensis* supplemented with *B. pseudomallei* exotoxin (Bt + E) (n =30). Live bacteria groups were inoculated with 5 x 10^4, 5 x 10^3 and 5 x 10^2 Colony-forming units (CFU) of either *B. pseudomallei* or *B. thailandensis* respectively. The exotoxin groups were inoculated with 50.0 µg; 25.0 µg and 12.5 µg exotoxin either alone or mixed with 5 x 10^4, 5 x 10^3 and 5 x 10^2 CFU of *B. thailandensis* respectively. Negative control groups (Cx) received sterile PBS (P^H 7.4) only. Clinical signs such as loss of body weight, change in appearance, behaviour and activity of the animal were monitored and recorded for 14 days. Lethal dose-50 (LD_{50}) and survival analysis were calculated. Organ bacterial burden, haematological, gross and histopathological analyses were conducted. There was significant (p<0.05) dose-dependent increase in the susceptibility of the BALB/c mice treated with the live bacteria (group Bp and Bt) following IN inoculation as compared to SC inoculation. This contrast with the observation in the groups of mice inoculated with exotoxins (E and Bt + E) in which those in the SC groups showed more susceptibility than IN inoculated groups. The most striking finding in this study was the enhancing of *B. thailandensis* pathogenicity following its supplementation with *B. pseudomallei* exotoxin isolated in this study. This was due to the fact that, although group Bt showed significant gross, histopathology, haematology body weight changes over time when compared to the uninfected control, mortality was not recorded, however, in group Bt + E, there was significant (p<0.05) morbidity and mortality when compared to the Bt and Cx groups. When these are combined with the findings of this study that showed the abundance of the exotoxin genes in *B. pseudomallei* while absent in *B. thailandensis*, suggested that the *B. pseudomallei* extracellular products (exotoxins) are necessary for virulence and pathogenicity observed in this bacterium. In conclusion, this study showed that the isolates contained exotoxin (phospholipase C) encoded by Plc-1, Plc-2 and plc-3 genes. The exotoxin plays an important role in the pathogenesis of melioidosis as it resulted in morbidity and mortality in mice model. Further studies should focus on cloning of this exotoxin in order to obtain the purest protein to be used in developing toxin (Toxoid) candidate for the use in countermeasures of melioidosis. Similarly, the exotoxin shall be evaluated in other models such as Nematodes models and *in vitro* cell lines in order to gain a wider understanding of its toxicity.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PERANAN EKSOTOKSIN *Burkholderia pseudomallei* DALAM PATOGENESIS MELIOIDOSIS MENGGUNAKAN MODEL MICE

Oleh

RABIU MUHAMMAD ALIYU

Jun 2016

Pengerusi : Noordin Mohamed Mustapha, PhD
Fakulti : Perubatan Veterinar

Burkholderia pseudomallei ialah sejenis bakteria Gram-negatif yang merupakan agen penyebab penyakit melioidosis, sejenis penyakit pelbagai sistem yang serius dan sering kali membawa maut yang boleh menjangkiti manusia dan haiwan. Di Malaysia, *B. pseudomallei* telah dipencil daripada pelbagai subjek dari negeri Pahang, Johor, Kuala Lumpur dan Kedah. Strain-strain *B. pseudomallei* diketahui boleh merembes pelbagai enzim ekstrasel (eksotoksin) yang mempunyai aktiviti-aktiviti maut yang berbeza seperti proteolisis, lipolisis, aktiviti-aktiviti hemolisis. Namun, hanya sedikit kajian telah dijalankan tentang ciri-ciri eksotoksin daripada isolat Malaysia. Selain itu, terdapat kekurangan maklumat tentang peranan isolat *B. pseudomallei* tempatan dengan/atau eksotoksin berkaitan dalam patogenesis melioidosis. Kajian ini bertujuan untuk mengenalpasti ciri-ciri molekular *B. pseudomallei* serta untuk mengkaji peranan eksotoksin dalam patogenesis melioidosis. Tiga isolat *B. pseudomallei*, strain UPM/2012/1017, UPM/2013/006 and UPM/2013/577 digunakan dalam kajian ini diperolehi dari kes-kes Melioidosis haiwan dibentangkan kepada Hospital Veterinar Universiti (UVH), Universiti Putra Malaysia (UPM). Isolat-isolat tersebut mula-mula telah dijalankan proses pencirian secara fenotip dan biokimia, kini diikuti oleh teknik pencirian molekular 16S rRNA PCR, penjujukan serta analisis jujukan mengunakan cara bioinformatik. Ujian-ujian bakteriologi dan biokimia telah mengesahkan semula isolat tersebut sebagai spesis *B. pseudomallei*. Penjujukan dan analisis filogenetik menunjukan isolat Malaysia berhubung rapat dengan strain *B. pseudomallei* K96243 and 1026b dari Thailand dan juga strain *B. pseudomallei* MSHR2543 dari Australia yang merupakan strain bervirulen tinggi serta dikenali secara global dan kerap dilaporkan dalam manusia, ini menunjukkan kemungkinan potensi zoonosis oleh strain-strain *B. pseudomallei* tempatan. Kajian ini telah cadangkan bahawa kewujudan meloidosis haiwan yang membawa maut disebabkan oleh strain-strain *B. pseudomallei* Malaysia yang secara filogenetiknya berhubung rapat dengan strain-strain berpatogenik dan bervirulen tinggi dari Thailand dan Australia.

Analisis gen virulen ekstrasel (eksotoksin) menunjukkan kehadiran tiga gen pengekodan bagi phospholipase C dan pengekodan gen keempat bagi protease. Pencirian lanjutan protein ekstrasel (eksotoksin) oleh analisis SDS-PAGE telah menunjukkan kehadiran enzim phospholipase C dengan berat molekular sebanyak 77 kDa. Selepas pencirian protein, satu assay *in vivo* mengunakan BALB/c mice telah dijalankan bagi menilai ketoksigenan protein ekstrasel yang dipencil. Sebanyak 130 BALB/c mencit telah digunakan dalam kajian ini. Mencit-mencit tersebut telah dibahagikan kepada 5 kumpulan rawatan: kawalan (Cx) (n =10), *B. pseudomallei* (Bp) hidup (n =30); *B. thailandensis* (Bt) hidup (n =30); *B. pseudomallei* eksotoksin (E) (n =30) and *B. thailandensis* ditambah dengan eksotoksin *B. pseudomallei* (Bt + E) (n =30). Kumpulan bakteria hidup masing-masing telah diinokulasi dengan 5 x 10^4, 5 X 10^3 and 5 x 10^2 unit pembentuk koloni (CFU) sama ada dengan *B. pseudomallei* atau *B. thailandensis*. Kumpulan eksotoksin masing-masing telah diinokulasi dengan 50.0 µg; 25.0 µg dan 12.5 µg eksotoksin sama ada sendirian atau dicampur dengan 5 x 10^4, 5 x 10^3 dan 5 x 10^2 CFU *B. thailandensis*. Kumpulan kawalan negatif hanya menerima PBS (P^H 7.4) steril. Petanda klinikal seperti susut berat badan, perubahan, tingkah laku dan aktiviti haiwan tersebut diperhati dan direkod selama 14 hari. Nilai LD_{50} and analisis kemandirian telah dikira. Analisis beban bakteria dalam organ, hematologi, pemeriksaan kasar dan histopatologi telah dijalankan. Terdapat perbezaan ketara (p<0.05) antara peningkatan bergantung pada dos dalam pendedahan mencit BALB/c dirawat dengan bakteria hidup (group Bp and Bt) selepas diinokulasi IN berbanding dengan inokulasi melalui SC. Ini bertentangan dengan apa yang dilihat dalam kumpulan tikus diinokulasi dengan eksotoksin (E and Bt + E) dimana kumpulan SC menunjukan lebih terdedah daripada kumpulan diinokulasi secara IN. Dapatan yang paling menarik ialah perubahan *B. thailandensis* menunjukan atribut virulen selepas ditambah dengan eksotoksin *B. pseudomallei* yang dipencil dalam kajian ini. Ini disebabkan oleh, walaupun mencit diinokulasi dengan *B. thailandensis* (group Bt) menunjukan perubahan ketara dalam patologi kasar, histopatologi, hematologi dan berat badan dalam tempoh masa bila dibanding dengan kumpulan kawalan, namun kematian tidak direkod, tetapi, dalam kumpulan yang diinokulasi dengan *B. thailandensis* ditambah dengan eksotoksin *B. pseudomallei* (group Bt + E), terdapat perbezaan ketara (p<0.05) dalam morbiditi and kematian apabila dibanding dengan kumpulan Bt and Cx. Hasil gabungan fakta bahawa terdapat banyak gen eksotoksin dalam *B. pseudomallei* namun tiada dalam *B. thailandensis* dalam kajian ini, membuktikan bahawa produk ekstrasel (eksotoksin) *B. pseudomallei* adalah penting untuk virulen dan kepatogenan diperhatikan dalam bakteria. Kesimpulannya, kajian ini menunjukan bahawa pencilan terkandung eksotoksin (Phospholipase C) pengekodan oleh Plc - 1, Plc - 2 dan PLC - 3 gen. Eksotoksin memainkan peranan penting patogenesis Melioidosis kerana ia mengakibatkan morbiditi dan kematian dalam model mice. Kajian mendatang sepatutnya fokus pada pengklonan eksotoksin ini bagi mendapat protein tulen bagi mengunakannya dalam membangunkan calon toksin (toksoid) sebagai cara menangani melioidosis. Selain itu, eksotoksin sepatutnya dinilai dalam model lain seperti model Nematoda dan garisan sel *in vitro* bagi mengetahui lebih meluas tentang ketoksikannya.

ACKNOWLEDGEMENTS

In the Name of Allah, the most Gracious, the most Merciful.

Let me start by giving thanks and sincere gratitude to Almighty Allah (S. W.A) for sparing my life, giving me strength and good health to successfully complete this study and guiding me through all aspect of life. To him I will say "ALHAMDU LILLAHI RABBIL ALAMIN".

I would like to express my sincere gratitude and appreciation to my project supervisors; Professor Noordin Mohamed Mustapha for his continued patience, guidance, support and a tremendous mentorship despite his tight and busy administrative schedules. I will also like to thank my research co-supervisors Assoc. Professor Dr. Zunita Zakaria and Assoc. Professor Dr. Siti Khairani Bejo for their advices, guidance and support. May Allah bless and reward them abundantly.

I wish to acknowledge, with deep gratitude and appreciation to my parents: Late Alhaji Muhammadu Aliyu and Hajjiya Asma'u Muhammad for bringing me up, their prayers, support and encouragement throughout my study. My hearty appreciation to my loving wife Nafisa Muhammad Tukur for her love, care and support throughout the journey.

I cannot forget the advice, encouragement, contribution and moral support of my senior colleagues Drs. Ibrahim Anka, Yusuf Yakubu, Faruk Bande, Adamu abdul, Sani Samaila, M. S. Abubakar. May Allah reward them abundantly.

I also wish to acknowledge the support, courage and prayers of my relatives, friends and well-wishers some of whom are Dr. Musa Isah, Mustapha Alkali, Dr. Ashiru Bello and Dr. Abubakar Amale.

Eventually, to all those who helped, either directly or indirectly towards the success of this study (too numerous to mention), Allah knows them all, may he reward them abundantly.

Praises be to Allah in the beginning and the end.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

BHI	Brain-heart infusion
Bp	*Burkholderia pseudomallei*
Bt	*Burkholderia thailandensis*
Bt + E	*Burkholderia thailandensis* + Exotoxin
bwt	Body weight
CFU	Colony-forming unit
CI	Confidence Interval
DNA	Deoxyribonucleic Acid
E	Exotoxin
IACUC	Institutional Animal Care and Usage committee
OECD	Organisation for Economic Co-operation and Development
µg	Microgram
µl	Microlitre
PI	Post-infection
UVH	University Veterinary Hospital

CHAPTER 1

INTRODUCTION

Burkholderia pseudomallei is aerobic, motile, gram-negative bacilli that are frequently isolated from damp soil, especially in rice paddies and stagnant water in the endemic regions of the world (Brett and Woods, 2000). This pathogen is non-fastidious and therefore able to grow relatively fast in conventional culture media such as nutrient agar, blood agar and macConkey agar. It can also selectively grow on Ashdown's medium with varied colonial morphology ranging from wrinkle to smooth and from creamy to orange with characteristic earthy odour (Gilad *et al.*, 2007a).

The organism infects both humans and animals through inhalation, ingestion and percutaneous inoculation of a wound, burn or abscess with contaminated water or wet soil to cause a disease called melioidosis. This disease is endemic primarily in regions of Southeast Asia and Northern Australia, but reported to occur sporadically in other regions in the world (Gilad *et al.*, 2007a). Previous studies review that the prevalence of *B. pseudomallei* has gone beyond these known areas of endemicity to other locations around the globe (Dance, 1991).

Documented epidemiological findings showed that soil and water are the major reservoirs of *B. pseudomallei*, however, various animals could also serve as maintenance reservoirs for the sustenance of epizootic infections (Gilad *et al.*, 2007b). Both domestic and wild animals that are susceptible to melioidosis including camels, cattle, horses, sheep, goats, cats, dogs, kangaroos, koalas, Alpacas, deer and few cases reported in birds (Cheng and Currie, 2005). Similarly, mouse, rats, chicken, guinea pigs and nematodes have been used as experimental animal models in studying pathogenesis of the disease (Cheng and Currie, 2005).

More recently, *B. pseudomallei* was listed under category B bioterrorism agent and reviewed to Tier 1 select agent by the United States Centre for Disease Control and Prevention (CDC). This is because of its increasing incidence, rate of infectivity and increasing fear of use as a potential biowarfare/bioterrorism agent, since its equine-adopted biotype (*B. mallei*) has previously been used in this manner (CDC, 2015). Melioidosis were reported in French troops who actively serve in French-Indochina war in 1950's and shortly after the world war II in American soldiers fought in Vietnam (White, 2003). Though many of the military cases are chronic, cases of reactivation after years of initial exposure were described (Currie *et al.*, 2000).

In Malaysia, the report of animal melioidosis has been published as far back as 1932 by Stolon and Fletcher of Medical Research Institute of then Malay state (Cheng and Currie, 2005). Similarly, human melioidosis has been reported in the states of Pahang, Johor, Kuala Lumpur and Kedah representing the eastern,

southern, middle and the northern region of the country respectively (Hassan *et al.*, 2010; Sapian *et al.*, 2012). Afterwards, cases of animal and human melioidosis were being continuously reported in both Peninsular and East Malaysia.

Although studies have shown that host immune responses play a significant role in the pathogenesis of melioidosis, however, the relative contribution of different bacterial product-such as exotoxin and endotoxin-in the pathogenesis and evasion of the immune system is still lacking. This dearth of knowledge might be partly responsible for inability to develop vaccines to protect humans and susceptible animal species despite several trials (Iliukhin *et al.*, 1999; Warawa and Woods, 2002; Peacock *et al.*, 2012).

Information on the virulence of *B. pseudomallei* is scanty (Cheng and Currie, 2005). The bacteria produced an array of extracellular products (protease, catalase, lipase, peroxidase, superoxidase dismutase, haemolysin, siderophore and lecithinase) which express a variety of toxic features, some of which include dermonecrotic effect, haemolytic and lethal toxicity. However, it is still unclear whether melioidosis is a toxin-mediated. Few studies have been conducted on the role of these exoproducts as virulent factors, among which only protease has been confirmed to aid in the pathogenesis of the disease (Sexton *et al.*, 1994).

Understanding the partial contribution of aforementioned bacterial exoproducts to the resultant pathogenesis, colonization and perseverance of the host immune system by this bacteria will pave the way in designing proper and effective therapeutic measures in controlling this endemic disease. Despite the endemicity of melioidosis in Malaysia, there is no effective vaccine available against the disease and the organism is not only resistant to most available and affordable antibiotics (Radu *et al.*, 2000; Podin *et al.*, 2014), but also the molecular mechanisms that underlie the pathway of disease progression is extremely unclear (Ulett *et al.*, 2001).

The organism expressed an array of biological/toxic activities, some of which include dermonecrotic effect, lethal toxicity, and various enzyme activities (protease, catalase, lipase, peroxidase, superoxidase dismutase, haemolysin, siderophore and lecithinase). However, it is still unclear whether melioidosis is toxin mediated.

In view of the importance of the disease and the aforementioned problems, there is the need for an in-depth investigation on the role of *B. pseudomallei* exotoxins in the pathogenic indices of melioidosis in animal models. There is also the need to examine if there is a significant difference between the isolates in respect of their pathogenicity.

Several studies highlighted the interactions of *B. pseudomallei* with the host immune system at cellular and molecular level (Razak and Ismail, 1982; Brett and

Woods, 2000; Wongratanacheewin *et al.*, 2004; Wikraiphat *et al.*, 2009). However, there is the need to explore further on this pathogenic interaction in relation to the ability of the bacteria to adhere, invade, survive and replicate within the susceptible host model.

Because of this gap of knowledge, this work might therefore be relevant in exploring this area of pathogenicity using animal model assay. It is also hoped that the data that will emanate from this study will elucidates the variation in relation to the pattern and severity of melioidosis among bacterial strains/isolates. This will help in designing appropriate control measures through understanding the mechanisms that interplay between the host and the bacterial exoproducts.

1.1 Research questions

1. Do *B. pseudomallei* and its non-pathogenic biotype *B. thailandensis* contain the same number of extracellular virulence determinant genes?
2. Is the non-virulence nature of *B. thailandensis* when compared to *B. pseudomallei* due to the absence of exotoxin?
3. Can the pathogenicity of *B. thailandensis* be enhanced when it is supplemented with exotoxin isolated from *B. pseudomallei*?
4. Is there any difference between the pathogenicity/lethality indexes (such as LD_{50}, survival values, haematology, gross pathology and histopathology) when BALB/c mice was inoculated via different routes with *B. pseudomallei*, its exotoxin, *B. thailandensis* and *B. thailandensis* supplemented with *B. pseudomallei* exotoxin?

1.2 Objectives

The objectives of the study are:

i. to characterise *B. pseudomallei* isolates from Malaysia using molecular techniques.
ii. to identify exotoxin genes among the *B. pseudomallei* strains characterised and *B. thailandensis* strain using molecular techniques.
iii. to isolate, characterise and compare exotoxin produced by *B. pseudomallei* strains and *B. thailandensis* strain.
iv. to investigate the role of exotoxin from *B. pseudomallei* in the pathogenesis of melioidosis in mice model.

1.3 Research hypothesis

Burkholderia thailandensis supplemented with exotoxin of *B. pseudomallei* will have an enhanced pathogenicity and will display virulence equivalent or close to that of *B. pseudomallei*.

REFERENCES

Alice, A.F., Lopez, C.S., Lowe, C.A., Ledesma, M.A., Crosa, J.H. (2006). Genetic and transcriptional analysis of the siderophore malleobactin biosynthesis and transport genes in the human pathogen Burkholderia pseudomallei K96243. *Journal of Bacteriology*. 188: 1551–1566.

Allwood, E.M., Devenish, R.J., Prescott, M., Adler, B., Boyce, J.D. (2011). Strategies for intracellular survival of Burkholderia pseudomallei. *Frontiers in Microbiology*. 2: 170.

Al-Marzooq, F., Imad, M.A., How, S.H., Kuan, Y.C. (2011). Development of multiplex real-time PCR for the rapid detection of five bacterial causes of community acquired pneumonia. *Tropical Biomedicine*. 28: 545–556.

Alouf, J.E. (2000). Bacterial toxins: methods and protocols., 1st: ed.: New Jersey.

American Society for Microbiology (2006). Sentinel laboratory guidelines for suspected agents of bioterrorism: Burkholderia mallei and B. pseudomallei.

Amornchai, P., Chierakul, W., Wuthiekanun, V., Mahakhunkijcharoen, Y., Phetsouvanh, R., Currie, B.J., Newton, P.N., Chau, N.v.V., Wongratanacheewin, S., Day, N.P.J. (2007). Accuracy of Burkholderia pseudomallei identification using the API 20NE system and a latex agglutination test. *Journal of Clinical Microbiology*. 45: 3774–3776.

Ang, Y.M. (2005). Neonatal meliodosis: very rare but be aware. *The Medical Journal of Malaysia*. 60: 99–102.

Antonov, V.A., Tkachenko, G.A., Altukhova, V. V, Zamaraev, V.S., Iliukhin, V.I., Trofimov, D.I. (2004). Use of PCR for identification of Burkholderia mallei. *Molekuliarnaia Genetika, Mikrobiologiia i Virusologiia*. 1: 12–17.

Anuntagool, N., Intachote, P., Wuthiekanun, V., White, N.J., Sirisinha, S. (1998). Lipopolysaccharide from nonvirulent Ara+ Burkholderia pseudomallei isolates is immunologically indistinguishable from lipopolysaccharide from virulent Ara- clinical isolates. *Clinical and Diagnostic Laboratory Immunology*. 5: 225–229.

Arjcharoen, S., Wikraiphat, C., Pudla, M., Limposuwan, K., Woods, D.E., Sirisinha, S., Utaisincharoen, P. (2007). Fate of a Burkholderia pseudomallei lipopolysaccharide mutant in the mouse macrophage cell line RAW 264.7: possible role for the O-antigenic polysaccharide moiety of lipopolysaccharide in internalization and intracellular survival. *Infection and Immunity*. 75: 4298–4304.

Ashdown, L.R. (1979). Identification of Pseudomonas pseudomallei in the clinical laboratory. *Journal of Clinical Pathology*. 32: 500–504.

Ashdown, L.R., Koehler, J.M. (1990). Production of hemolysin and other extracellular enzymes by clinical isolates of Pseudomonas pseudomallei. *Journal of Clinical Microbiology*. 28: 2331–2334.

Barker, A.P., Vasil, A.I., Filloux, A., Ball, G., Wilderman, P.J., Vasil, M.L. (2004). A novel extracellular phospholipase C of Pseudomonas aeruginosa is required for phospholipid chemotaxis. *Molecular Microbiology*. 53: 1089–1098.

Boddey, J.A., Flegg, C.P., Day, C.J., Beacham, I.R., Peak, I.R. (2006). Temperature-regulated microcolony formation by Burkholderia pseudomallei requires pilA and enhances association with cultured human cells. *Infection and Immunity*. 74: 5374–81.

Bradford, M.M. (1976). Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. *Analytical Biochemistry*. 72: 248–254.

Brett, P., Deshazer, D., Woods, D. (1998). Burkholderia thailandensis sp. nov., a Burkholderia pseudomallei like species. *Journal of Clinical Microbiology*. 48: 317–320.

Brett, P., Woods, D. (2000). Pathogenesis of and immunity to melioidosis. *Acta Tropica*. 74: 201–210.

Brett, P.J., Deshazer, D., Woods, D.E. (1997). Characterization of Burkholderia pseudomallei and Burkholderia pseudomallei-like strains. *Epidermiology and Infections*. 118: 137–148.

Brilhante, R.S.N., Bandeira, T.J.P.G., Cordeiro, R.A., Grangeiro, T.B., Lima, R.A.C., Ribeiro, J.F., Castelo-Branco, D.S.C.M., Rodrigues, J.L.N., Coelho, I.C.B., Magalhães, F.G., Rocha, M.F.G., Sidrim, J.J.C. (2012). Clinical-epidemiological features of 13 cases of melioidosis in Brazil. *Journal of Clinical Microbiology*. 50: 3349–3352.

Brook, M.D., Currie, B., Desmarchelier, P.M. (1997). Isolation and identification of Burkholderia pseudomallei from soil using selective culture techniques and the polymerase chain reaction. *Journal of Applied Microbiology*. 82: 589–596.

Burkholder, W.H. (1950). Sour skin, a bacterial rot of onion bulbs. *Phytopathology*. 40: 115–118.

CDC (2015). Conclusion of select agent inquiry into Burkholderia pseudomallei release at Tulane national primate research center.

Chaiyaroj, S.C., Kotrnon, K., Koonpaew, S., Anantagool, N., White, N.J., Sirisinha, S. (1999). Differences in genomic macrorestriction patterns of arabinose-positive (Burkholderia thailandensis) and arabinose-negative Burkholderia pseudomallei. *Microbiology and Immunology*. 43: 625–630.

Chandni, R. (2013). Melioidosis: The Great Mimicker, 10th: ed.: Chennai.

Chantratita, N., Wuthiekanun, V., Boonbumrung, K., Tiyawisutsri, R., Vesaratchavest, M., Limmathurotsakul, D., Chierakul, W., Wongratanacheewin, S., Pukritiyakamee, S., White, N.J., Day, N.P.J., Peacock, S.J. (2007a). Biological relevance of colony morphology and phenotypic switching by Burkholderia pseudomallei. *Journal of*

Bacteriology. 189: 807–817.

Chantratita, N., Wuthiekanun, V., Limmathurotsakul, D., Thanwisai, A., Chantratita, W., Day, N.P.J., Peacock, S.J. (2007b). Prospective clinical evaluation of the accuracy of 16S rRNA real-time PCR assay for the diagnosis of melioidosis. *The American Journal of Tropical Medicine and Hygiene*. 77: 814–817.

Chen, Y., Lin, H., Pan, N., Chien, S., Chen, Y. (2002). Comparison of 16S RNA, flagella and type III secretion gene PCR primer sets for diagnosis of Ara-Burkholderia pseudomallei in paddy soil. *Journal of Biomedical Laboratory Science*. 14: 95–100.

Chen, Y., Wong, J., Sun, G.W., Liu, Y., Tan, G.-Y.G., Gan, Y.-H. (2011). Regulation of type VI secretion system during Burkholderia pseudomallei infection. *Infection and Immunity*. 79: 3064–3073.

Chen, Y.-L., Lin, Y.-C., Chen, Y.-S., Chen, S.-C., Liu, Y.-M., Tseng, I.-L., Chiang, C.-S., Lin, H.-H., Mu, J.-J. (2013). Characterisation of predominant molecular patterns of Burkholderia pseudomallei in Taiwan. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 107: 165–169.

Chen, Y.-S., Lin, H.-H., Mu, J.-J., Chiang, C.-S., Chen, C.-H., Buu, L.-M., Lin, Y.E., Chen, Y.-L. (2010). Distribution of melioidosis cases and viable Burkholderia pseudomallei in soil: evidence for emerging melioidosis in Taiwan. *Journal of Clinical Microbiology*. 48: 1432–1434.

Cheng, A.C., Currie, B.J. (2005). Melioidosis: epidemiology, pathophysiology, and management. *Clinical Microbiology Reviews*. 18: 383–416.

Cheng, A.C., Hanna, J.N., Norton, R., Hills, S.L., Davis, J., Krause, V.L., Dowse, G., Inglis, T.J., Currie, B.J. (2003). Melioidosis in northern Australia, 2001-02. *Communicable Diseases Intelligence*. 27: 272–277.

Cheng, A.C., O'Brien, M., Freeman, K., Lum, G., Currie, B.J. (2006). Indirect hemagglutination assay in patients with melioidosis in northern Australia. *The American Journal of Tropical Medicine and Hygine*. 74: 330–334.

Chenthamarakshan, V., Vadivelu, J., Puthucheary, S.D. (2001). Detection of immunoglobulins M and G using culture filtrate antigen of Burkholderia pseudomallei. *Diagnostic Microbiology and Infectious Disease*. 39: 1–7.

Chieng, S., Carreto, L., Nathan, S. (2012). Burkholderia pseudomallei transcriptional adaptation in macrophages. *BMC Genomics*. 13: 328.

Chirakul, S., Bartpho, T., Wongsurawat, T., Taweechaisupapong, S., Sermswan, R.W. (2014). Characterization of BPSS1521 (bprD), a regulator of Burkholderia pseudomallei virulence gene expression in the mouse model. *PloS One*. 9: e104313.

Choh, L.-C., Ong, G.-H., Vellasamy, K.M., Kalaiselvam, K., Kang, W.-T., Al-Maleki, A.R., Mariappan, V., Vadivelu, J. (2013). Burkholderia vaccines: are we moving forward? *Frontiers in Cellular and Infection Microbiology*. 3: 1–18.

Chua, K.H., See, K.H., Thong, K.L., Puthucheary, S.D. (2010). DNA fingerprinting of human isolates of Burkholderia pseudomallei from different geographical regions of Malaysia. *Tropical Biomedicine*. 27: 517–524.

Chua, K.H., See, K.H., Thong, K.L., Puthucheary, S.D. (2011). SpeI restriction enzyme displays greater discriminatory power than XbaI enzyme does in a pulsed-field gel electrophoresis study on 146 clinical Burkholderia pseudomallei isolates. *Japanese Journal of Infectious Diseases*. 64: 228–233.

Chua, K.L., Chan, Y.Y., Gan, Y.H. (2003). Flagella are virulence determinants of Burkholderia pseudomallei. *Infection and Immunity*. 71: 1622–1629.

Chuaygud, T., Tungpradabkul, S., Sirisinha, S., Chua, K.L., Utaisincharoen, P. (2008). A role of Burkholderia pseudomallei flagella as a virulent factor. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 102: S140–144.

Collier, R.J. (1975). Diphtheria toxin: mode of action and structure. *Bacteriological Reviews*. 39: 54–85.

Colling, M., Nigg, C., Heckly, R.J. (1958). Toxins of Pseudomonas pseudomallei. I. Production in vitro. *Journal of Bacteriology*. 76: 422–426.

Cooper, A., Williams, N.L., Morris, J.L., Norton, R.E., Ketheesan, N., Schaeffer, P.M. (2013). ELISA and immuno-polymerase chain reaction assays for the sensitive detection of melioidosis. *Diagnostic Microbiology and Infectious Disease*. 75: 135–8.

Corea, E., Thevanesam, V., Perera, S., Jayasinghe, I., Ekanayake, A., Masakorala, J., Inglis, T.J.J. (2012). Melioidosis in Sri Lanka: an emerging infection. *Sri Lankan Journal of Infectious Diseases*. 2: 2–8.

Couto, M.S., Cordeiro, R. de A., Rocha, M.F.G., Grangeiro, T.B., Leitão Junior, N.P., Bandeira, T. de J.P.G., Sidrim, J.J.C., Brilhante, R.S.N. (2009). A diagnosis of Burkholderia pseudomallei directly in a bronchoalveolar lavage by polymerase chain reaction. *Diagnostic Microbiology and Infectious Disease*. 65: 73–75.

Currie, B.J. (2003). Melioidosis: an important cause of pneumonia in residents of and travellers returned from endemic regions. *European Respiratory Journal*. 22: 542–550.

Currie, B.J., Fisher, D.A., Anstey, N.M., Jacups, S.P. (2000). Melioidosis: acute and chronic disease, relapse and re-activation. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 94: 301–304.

Currie, B.J., Ward, L., Cheng, A.C. (2010). The epidemiology and clinical spectrum of melioidosis: 540 cases from the 20 year Darwin prospective study. *PLoS Neglected Tropical Diseases*. 4: e900.

Dance, D.A., King, C., Aucken, H., Knott, C.D., West, P.G., Pitt, T.L. (1992). An outbreak of melioidosis in imported primates in Britain. *The Veterinary*

Record. 130: 525–9.

Dance, D.A.B. (1991). Melioidosis: the Tip of the Iceberg? *Clinical Microbiology Reviews*. 4: 52–64.

Dance, D.A.B. (2000a). Melioidosis as an emerging global problem. *Acta Tropica*. 74: 115–119.

Dance, D.A.B. (2000b). Ecology of Burkholderia pseudomallei and the interactions between environmental Burkholderia spp. and human-animal hosts. *Acta Tropica*. 74: 159–168.

De Smet, B., Sarovich, D.S., Price, E.P., Mayo, M., Theobald, V., Kham, C., Heng, S., Phe, T., Holden, M.T.G., Parkhill, J., Peacock, S.J., Spratt, B.G., Jacobs, J., Vandamme, P., Currie, B.J. (2014). Whole genome sequencing confirms that Burkholderia pseudomallei multilocus sequence types common to both Cambodia and Australia are due to homoplasy. *Journal of Clinical Microbiology*. 53: 323–326.

Deris, Z.Z., Hasan, H., Noor, M., Suraiya, S. (2005). Clinical characteristics and outcomes of bacteraemic melioidosis in a teaching hospital in a northeastern state of Malaysia : a five-year review. *The Journal of Infection in Developing Countries*. 4: 430–435.

DeShazer, D., Brett, P.J., Burtnick, M.N., Woods, D.E. (1999). Molecular Characterization of Genetic Loci Required for Secretion of Exoproducts in Burkholderia pseudomallei. *Journal of bacteriology*. 181: 4661–4664.

Dharakul, T., Songsivilai, S., Viriyachitra, S., Luangwedchakarn, V., Tassaneetritap, B., Chaowagul, W. (1996). Detection of Burkholderia pseudomallei DNA in patients with septicemic melioidosis. *Journal of Clinical Microbiology*. 34: 609–614.

Dharakul, T., Tassaneetrithep, B., Trakulsomboon, S. (1999). Phylogenetic Analysis of Ara + and Ara − Burkholderia pseudomallei isolates and development of a multiplex PCR procedure for rapid discrimination between the two biotypes. *Journal of Clinical Microbiology*. 37: 1906–1912.

Dodin, A., Galimand, M. (1986). [Origin, course and recession of an infectious disease, melioidosis, in temperate countries]. *Archives de l'Institut Pasteur de Tunis*. 63: 69–73.

Essex-Lopresti, A.E., Boddey, J.A., Thomas, R., Smith, M.P., Hartley, M.G., Atkins, T., Brown, N.F., Tsang, C.H., Peak, I.R.A., Hill, J., Beacham, I.R., Titball, R.W. (2005). A type IV pilin, pilA, contributes to adherence of Burkholderia pseudomallei and virulence in vivo. *Infection and Immunity*. 73: 1260–1264.

Fisher, N.A., Ribot, W.J., Applefeld, W., DeShazer, D. (2012). The Madagascar hissing cockroach as a novel surrogate host for Burkholderia pseudomallei, B. mallei and B. thailandensis. *BMC Microbiology*. 12: 117.

French, C.T., Toesca, I.J., Wu, T.-H., Teslaa, T., Beaty, S.M., Wong, W., Liu, M.,

Schröder, I., Chiou, P.-Y., Teitell, M.A., Miller, J.F. (2011). Dissection of the Burkholderia intracellular life cycle using a photothermal nanoblade. *Proceedings of the National Academy of Sciences of the United States of America*. 108: 12095–12100.

Gauthier, Y.P., Hagen, R.M., Brochier, G.S., Neubauer, H., Splettstoesser, W.D., Finke, E., Vidal, D.R. (2001). Study on the pathophysiology of experimental Burkholderia pseudomallei infection in mice. *FEMS Immunology and Medical Microbiology*. 30: 53–63.

Gauthier, Y.P., Thibault, F.M., Paucod, J.C., Vidal, D.R. (2000). Protease production by Burkholderia pseudomallei and virulence in mice. *Acta Tropica*. 74: 215–520.

Gee, J.E., Sacchi, C.T., Glass, M.B., De, B.K., Weyant, R.S., Levett, P.N., Whitney, A.M., Alex, R., Hoffmaster, A.R., Popovic, T. (2003). Use of 16S rRNA gene sequencing for rapid identification and differentiation of Burkholderia pseudomallei and B. mallei. *Journal of Clinical Microbiology*. 41: 4647–4652.

Gilad, J., Harary, I., Dushnitsky, T., Schwartz, D., Amsalem, Y. (2007a). Burkholderia mallei and Burkholderia pseudomallei as bioterrorism agents: national aspects of emergency preparedness. *The Israel Medical Association Journal*. 9: 499–503.

Gilad, J., Schwartz, D., Amsalem, Y. (2007b). Clinical features and laboratory diagnosis of infection with the potential bioterrorism agents Burkholderia mallei and Burkholderia pseudomallei. *International Journal of Biomedical Science*. 3: 144–152.

Glass, M.B., Popovic, T. (2005). Preliminary evaluation of the API 20NE and RapID NF Plus systems for rapid identification of Burkholderia pseudomallei and B. mallei. *Journal of Clinical Microbiology*. 43: 479–483.

Godoy, D., Randle, G., Simpson, A.J., Aanensen, M., Pitt, T.L., Kinoshita, R., Brian, G., Aanensen, D.M., Spratt, B.G. (2003). Multilocus sequence typing and evolutionary relationships among the causative agents of melioidosis and glanders, Burkholderia pseudomallei and Burkholderia mallei. *Journal of Clinical Microbiology*. 41: 2068–2079.

Groleau, M., Dekimpe, V., Déziel, E. (2007). Burkholderia diversity and versatility: an Inventory of the extracellular products. *Journal of Microbiology and Biotechnology*. 17: 1407–1429.

Haase, A., Brennan, M., Barrett, S., Wood, Y., Huffam, S., O'Brien, D., Currie, B. (1998). Evaluation of PCR for diagnosis of melioidosis. *Journal of Clinical Microbiology*. 36: 1039–1041.

Haase, A., Janzen, J., Barrett, S., Currie, B. (1997). Toxin production by Burkholderia pseudomallei strains and correlation with severity of melioidosis. *Journal of Medical Microbiology*. 46: 557–563.

Haase, A., Melder, A., Smith-Vaughan, H., Kemp, D., Currie, B. (1995). RAPD analysis of isolates of Burkholderia pseudomallei from patients with

recurrent melioidosis. *Epidemiology and Infections*. 115: 115–121.

Hagen, R.M., Frickmann, H., Elschner, M., Melzer, F., Neubauer, H., Gauthier, Y.P., Racz, P., Poppert, S. (2001). Rapid identification of Burkholderia pseudomallei and Burkholderia mallei by fluorescence in situ hybridization (FISH) from culture and paraffin-embedded tissue samples. *International Journal of Medical Microbiology*. 301: 585–590.

Hagen, R.M., Gauthier, Y.P., Sprague, L.D., Vidal, D.R., Zysk, G., Finke, E.-J., Neubauer, H. (2002). Strategies for PCR based detection of Burkholderia pseudomallei DNA in paraffin wax embedded tissues. *Molecular Pathology*. 55: 398–400.

Halder, D., Zainal, N., Wah, C.M., Haq, J.A. (1998). Neonatal meningitis and septicaemia caused by Burkholderia pseudomallei. *Annals of Tropical Paediatrics*. 18: 161–164.

Hall, T. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*. 41: 95–98.

Harley, V.S., Dance, D.A., Tovey, G., McCrossan, M. V, Drasar, B.S. (1998). An ultrastructural study of the phagocytosis of Burkholderia pseudomallei. *Microbios*. 94: 35–45.

Häse, C.C., Finkelstein, R.A. (1993). Bacterial extracellular zinc-containing metalloproteases. *Microbiological Reviews*. 57: 823–837.

Hassan, M.R.A., Pani, S.P., Peng, N.P., Voralu, K., Vijayalakshmi, N., Mehanderkar, R., Aziz, N.A., Michael, E. (2010). Incidence, risk factors and clinical epidemiology of melioidosis: a complex socio-ecological emerging infectious disease in the Alor Setar region of Kedah, Malaysia. *BMC Infectious Diseases*. 10: 302.

Hasselbring, B.M., Patel, M.K., Schell, M.A. (2011). Dictyostelium discoideum as a model system for identification of Burkholderia pseudomallei virulence factors. *Infection and Immunity*. 79: 2079–2088.

HäuBler, S., Nimtz, M., Domke, T., Wray, V., Steinmetz, I., Häußler, S., Nimtz, M., Domke, T., Wray, V., Steinmetz, I., Häußler, S., Nimtz, M., Domke, T., Wray, V., Steinmetz, I., HäuBler, S., Nimtz, M., Domke, T., Wray, V., Steinmetz, I., Häußler, S. (1998). Purification and Characterization of a Cytotoxic Exolipid of Burkholderia pseudomallei. *Infection and Immunity*. 66: 1588–1593.

Haynes, W.C. (1957). Bergey's Manual of Determinative Bacteriology, 7th: ed.: Baltimore.

Heckly, R.J. (1964). Differentiation of exotoxin and other biologically active substances in Pseudomonas Pseudomallei filtrates. *Journal of Bacteriology*. 88: 1730–1736.

Heckly, R.J., Nigg, C. (1958). Toxins of Pseudomonas pseudomallei II: Characterization. *Journal of Bacteriology*. 76: 427–436.

Heng, B.H., Goh, K.T., Yap, E.H., Loh, H., Yeo, M. (1998). Epidemiological surveillance of melioidosis in Singapore. *Annals of the Academy of Medicine, Singapore*. 27: 478–84.

Ho, C.-C., Lau, C.C.Y., Martelli, P., Chan, S.-Y., Tse, C.W.S., Wu, A.K.L., Yuen, K.-Y., Lau, S.K.P., Woo, P.C.Y. (2011). Novel pan-genomic analysis approach in target selection for multiplex PCR identification and detection of Burkholderia pseudomallei, Burkholderia thailandensis, and Burkholderia cepacia complex species: a proof-of-concept study. *Journal of Clinical Microbiology*. 49: 814–821.

Holden, M.T.G., Titball, R.W., Peacock, S.J., Cerdeño-Tárraga, A.M., Atkins, T., Crossman, L.C., Pitt, T., Churcher, C., Mungall, K., Bentley, S.D., Sebaihia, M., Thomson, N.R., Bason, N., Beacham, I.R., Brooks, K., Brown, K.A., Brown, N.F., Challis, G.L., Cherevach, I., Chillingworth, T., Cronin, A., Crossett, B., Davis, P., DeShazer, D., Feltwell, T., Fraser, A., Hance, Z., Hauser, H., Holroyd, S., Jagels, K., Keith, K.E., Maddison, M., Moule, S., Price, C., Quail, M.A., Rabbinowitsch, E., Rutherford, K., Sanders, M., Simmonds, M., Songsivilai, S., Stevens, K., Tumapa, S., Vesaratchavest, M., Whitehead, S., Yeats, C., Barrell, B.G., Oyston, P.C.F., Parkhill, J. (2004). Genomic plasticity of the causative agent of melioidosis, Burkholderia pseudomallei. *Proceedings of the National Academy of Sciences of the United States of America*. 101: 14240–14245.

Hsueh, P.R., Teng, L.J., Lee, L.N., Yu, C.J., Yang, P.C., Ho, S.W., Luh, K.T. (2001). Melioidosis: an emerging infection in Taiwan? *Emerging Infectious Diseases*. 7: 428–433.

Iglewski, B.H., Liu, P. V, Kabat, D. (1977). Mechanism of action of Pseudomonas aeruginosa exotoxin A: Adenosine diphosphate-ribosylation of mammalian elongation factor 2 in vitro and in vivo. *Infection and Immunity*. 15: 138–44.

Iliukhin, V.I., Kislichkin, N.N., Merinova, L.K., Riapis, L.A., Denisov, I.I., Farber, S.M., Kisilichkina, O.I. (1999). [The outlook for the development of live vaccines for the prevention of melioidosis.]. *Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii*. 3: 52–55.

Inglis, T., Merritt, A., Chidlow, G., Aravena-Roman, M., Harnett, G. (2005). Comparison of diagnostic laboratory methods for identification of Burkholderia pseudomallei. *Journal of Clinical Microbiology*. 43: 2201–2206.

Inglis, T.J., Chiang, D., Lee, G.S., Chor-Kiang, L. (1998). Potential misidentification of Burkholderia pseudomallei by API 20NE. *Pathology*. 30: 62–64.

Inglis, T.J., Healy, P.E., Fremlin, L.J., Golledge, C.L. (2012). Use of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry analysis for rapid confirmation of Burkholderia pseudomallei in septicemic melioidosis. *The American Journal of Tropical Medicine and Hygine*. 86: 1039–1042.

Inglis, T.J.J., Aravena-Roman, M., Ching, S., Croft, K., Wuthiekamm, V., A., Mee, B.J. (2003). Cellular fatty acid profile distinguishes Burkholderia pseudomallei from avirulent Burkholderia thailandensis. *Journal of Clinical Microbiology*. 41: 4812–4814.

Inglis, T.J.J., Rigby, P., Robertson, T.A., Dutton, N.S., Henderson, M., Chang, B.J., Al, I.E.T. (2000). Interaction between Burkholderia pseudomallei and Acanthamoeba Species Results in Coiling Phagocytosis , Endamebic Bacterial Survival , and Escape. *Infection and Immunity*. 68: 1681–1686.

Inglis, T.J.J., Sagripanti, J.-L. (2006). Environmental factors that affect the survival and persistence of Burkholderia pseudomallei. *Applied and Environmental Microbiology*. 72: 6865–6875.

Ismail, G., Mohamed, R., Rohana, S., Sharifah, H.S., Embi, N. (1991). Antibody to Pseudomonas pseudomallei exotoxin in sheep exposed to natural infection. *Veterinary Microbiology*. 27: 277–282.

Ismail, G., Smith, C.J., Fast, N. (1987). Enzyme immunoassay for the detection of antibody to Pseudomonas pseudomallei exotoxin in mice. *FEMS Microbiology Letters*. 40: 27–31.

Jager, C.P.C. de, Wijk, P.T.L. van, Mathoera, R.B., Jongh-leuvenink, J. de, Poll, T. van der, Wever, P.C. (2010). Lymphocytopenia and neutrophil-lymphocyte count ratio predict bacteremia better than conventional infection markers in an emergency care unit. *Critical Care*. 14: R192.

Jones, A., Beveridge, T., Woods, D. (1996). Intracellular survival of Burkholderia pseudomallei. *Infection and Immunity*. 64: 782–790.

Jones, A.L., DeShazer, D., Woods, D.E. (1997). Identification and characterization of a two-component regulatory system involved in invasion of eukaryotic cells and heavy-metal resistance in Burkholderia pseudomallei. *Infection and Immunity*. 65: 4972–4977.

Jones, S.M., Ellis, J.F., Russell, P., Griffin, K.F., Oyston, P.C.F. (2002). Passive protection against Burkholderia pseudomallei infection in mice by monoclonal antibodies against capsular polysaccharide, lipopolysaccharide or proteins. *Journal of Medical Microbiology*. 51: 1055–62.

Joseph, M.M., Menon, J., Robinson, F., Rahman, M.S. (2015). Clinical manifestations, diagnosis and treatment of melioidosis. *IOSR Jouurnal of Pharmacology*. 5: 13–19.

Kaestli, M., Richardson, L.J., Colman, R.E., Tuanyok, A., Price, E.P., Bowers, J.R., Mayo, M., Kelley, E., Seymour, M.L., Sarovich, D.S., Pearson, T., Engelthaler, D.M., Wagner, D.M., Keim, P.S., Schupp, J.M., Currie, B.J. (2012a). Comparison of TaqMan PCR assays for detection of the melioidosis agent Burkholderia pseudomallei in clinical specimens. *Journal of Clinical Microbiology*. 50: 2059–2062.

Kaestli, M., Schmid, M., Mayo, M., Rothballer, M., Harrington, G., Richardson, L., Hill, A., Hill, J., Tuanyok, A., Keim, P., Hartmann, A., Currie, B.J. (2012b). Out of the ground: aerial and exotic habitats of the melioidosis bacterium

Burkholderia pseudomallei in grasses in Australia. *Environmental Microbiology*. 14: 2058–2070.

Kanai, K., Kondo, E. (1994). Recent advances in biomedical sciences of Burkholderia pseudomallei (Basonym: Pseudomonas pseudomallei). *Japanese Journal of Medical science and Biology*. 47: 1–45.

Kao, C.M., Chen, S.C., Chen, Y.S., Lin, H.M., Chen, Y.L. (2003). Detection of Burkholderia pseudomallei in rice fields with PCR-based technique. *Folia Microbiology*. 48: 521–524.

Koonpaew, S., Ubol, M.N., Sirisinha, S., White, N.J., Chaiyaroj, S.C. (2000). Genome fingerprinting by pulsed-field gel electrophoresis of isolates of Burkholderia pseudomallei from patients with melioidosis in Thailand. *Acta Tropica*. 74: 187–191.

Korbsrisate, S., Suwanasai, N., Ezaki, T., Kawamura, Y., Sarasombath, S. (1999). Cloning and characterization of a nonhemolytic phospholipase C gene from Burkholderia pseudomallei. *Journal of Clinical Microbiology*. 37: 3742–3745.

Korbsrisate, S., Tomaras, A.P., Damnin, S., Ckumdee, J., Srinon, V., Lengwehasatit, I., Vasil, M.L., Suparak, S. (2007). Characterization of two distinct phospholipase C enzymes from Burkholderia pseudomallei. *Microbiology*. 153: 1907–1915.

Kunakorn, M., Markham, R.B. (1995). Clinically practical seminested PCR for Burkholderia pseudomallei quantitated by enzyme immunoassay with and without solution hybridization. *Journal of Clinical Microbiology*. 33: 2131–2135.

Kunakorn, M., Raksakait, K., Sethaudom, C., Sermswan, R.W., Dharakul, T. (2000). Comparison of three PCR primer sets for diagnosis of septicemic melioidosis. *Acta Tropica*. 74: 247–251.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*. 227: 680–685.

Lafontaine, E.R., Zimmerman, S.M., Shaffer, T.L., Michel, F., Gao, X., Hogan, R.J. (2013). Use of a safe, reproducible, and rapid aerosol delivery method to study infection by Burkholderia pseudomallei and Burkholderia mallei in mice. *PloS One*. 8: e76804.

Leakey, A.K., Ulett, G.C., Hirst, R.G. (1998). BALB/c and C57Bl/6 mice infected with virulent Burkholderia pseudomallei provide contrasting animal models for the acute and chronic forms of human melioidosis. *Microbial Pathogenesis*. 24: 269–275.

Lee, M.A., Liu, Y. (2000). Sequencing and characterization of a novel serine metalloprotease from Burkholderia pseudomallei. *FEMS Microbiology Letters*. 192: 67–72.

Lee, S.-H., Chong, C.-E., Lim, B.-S., Chai, S.-J., Sam, K.-K., Mohamed, R., Nathan, S. (2007). Burkholderia pseudomallei animal and human isolates

from Malaysia exhibit different phenotypic characteristics. *Diagnostic Microbiology and Infectious Disease*. 58: 263–270.

Lee, S.H., Nathan, S. (2013). Burkholderia pseudomallei: An update on disease, virulence and host interaction. *Malaysian Applied Biology*. 42: 1–14.

Lee, S.-H., Ooi, S.-K., Mahadi, N.M., Tan, M.-W., Nathan, S. (2011). Complete killing of Caenorhabditis elegans by Burkholderia pseudomallei is dependent on prolonged direct association with the viable pathogen. *PloS One*. 6: e16707.

Lee, Y.H., Chen, Y., Ouyang, X., Gan, Y.-H. (2010). Identification of tomato plant as a novel host model for Burkholderia pseudomallei. *BMC Microbiology*. 10: 28.

Leelayuwat, C., Romphruk, A., Lulitanond, A., Trakulsomboon, S., Thamlikitkul, V. (2000). Genotype analysis of Burkholderia pseudomallei using randomly amplified polymorphic DNA (RAPD): indicative of genetic differences amongst environmental and clinical isolates. *Acta Tropica*. 77: 229–237.

Lew, A.E., and Desmarchelier, P.M. (1994). Detection of Pseudomonas pseudomallei by PCR and hybridization. *Journal of Clinical Microbiology*. 32: 1326–1332.

Li, X., Hayward, A.C. (1994). Bacterial whole cell protein profiles of the rRNA group 11 pseudomonads. *Journal of Applied Bacteriology*. 77: 308–318.

Limmathurotsakul, D., Dance, D.A.B., Wuthiekanun, V., Kaestli, M., Mayo, M., Warner, J., Wagner, D.M., Tuanyok, A., Wertheim, H., Yoke Cheng, T., Mukhopadhyay, C., Puthucheary, S., Day, N.P.J., Steinmetz, I., Currie, B.J., Peacock, S.J. (2013). Systematic review and consensus guidelines for environmental sampling of Burkholderia pseudomallei. *PLoS Neglected Tropical Diseases*. 7: e2105.

Limmathurotsakul, D., Peacock, S.J. (2011). Melioidosis: a clinical overview. *British Medical Bulletin*. 99: 125–139.

Limmathurotsakul, D., Wongratanacheewin, S., Teerawattanasook, N., Wongsuvan, G., Chaisuksant, S., Chetchotisakd, P., Chaowagul, W., Day, N.P.J., Peacock, S.J. (2010). Increasing incidence of human melioidosis in Northeast Thailand. *The American Journal of Tropical Medicine and Hygiene*. 82: 1113–1117.

Liu, P. V. (1957). Survey of hemolysin production among species of pseudomonads. *Journal of Bacteriology*. 74: 718–727.

Liu, Y., Wang, D., Ã, E.U.H.Y.A.P., Yap, E.P.H., Lee, M. (2002). Identification of a novel repetitive DNA element and its use as a molecular marker for strain typing and discrimination of ara- from ara+ Burkholderia pseudomallei isolates. *Journal of Medical Microbiology*. 51: 76–82.

Locht, C. (2012). Bacterial pathogenesis : molecular and cellular mechanisms: Norfolk, UK : Caister Academic Press.

Lowe, P., C. Engler, A., Norton., R. (2002). Comparison of automated and nonautomated systems for identification of Burkholderia pseudomallei. *Journal of Clinical Microbiology*. 40: 4625–4627.

Lowe, W., March, J.K., Bunnell, A.J., Neill, L.O., Robison, R.A. (2007). PCR-based methodologies used to detect and differentiate the Burkholderia pseudomallei complex : B. pseudomallei, B. mallei, and B. thailandensis. *Current Issues in Molecular Biology*. 16: 23–54.

Masoud, H., Ho, M., Schollaardt, T., Perry, M.B. (1997). Characterization of the capsular polysaccharide of Burkholderia (Pseudomonas) pseudomallei 304b. *Journal of bacteriology*. 179: 5663–5669.

Massey, S., Yeager, L.A., Blumentritt, C.A., Vijayakumar, S., Sbrana, E., Peterson, J.W., Brasel, T., LeDuc, J.W., Endsley, J.J., Torres, A.G. (2014). Comparative Burkholderia pseudomallei natural history virulence studies using an aerosol murine model of infection. *Scientific Reports*. 4: 4305.

Matsuura, M. (1996). Biological activities of lipopolysaccharide of Burkholderia (Pseudomonas) pseudomallei. *FEMS Microbiology Letters*. 137: 79–83.

Merritt, A., Inglis, T.J.J., Chidlow, G., Harnett, G. (2006). PCR-Based Identification of Burkholderia pseudomallei. *Revista do Instituto de Medicina Tropical de São Paulo*. 48: 239–244.

Miller, R.M., Tomaras, A.P., Barker, A.P., Voelker, D.R., Chan, E.D., Vasil, A.I., Vasil, M.L. (2008). Pseudomonas aeruginosa twitching motility-mediated chemotaxis towards phospholipids and fatty acids: Specificity and metabolic requirements. *Journal of Bacteriology*. 190: 4038–4049.

Mokhtar, S.M., Jalil, L.A. (2014). Melioidosis presenting as osteomyelitis of the mandible. *Journal of Oral and Maxillofacial Surgery, Medicine and Pathology*. 26: 359–361.

Mollaret, H.H. (1988). "L"affaire du jardin des plantes' ou comment la me ´lioı ¨dose fit son apparition en France. *Médecine et Maladies Infectieuses*. 18: 643–654.

Morgulis, A., Coulouris, G., Raytselis, Y., Madden, T.L., Agarwala, R., Schäffer, A.A. (2008). Database indexing for production MegaBLAST searches. *Bioinformatics (Oxford, England)*. 24: 1757–1764.

Mustafa, M.I., Al-Marzooq, F., How, S.H., Kuan, Y.C., Ng, T.H. (2011). The use of multiplex real-time PCR improves the detection of the bacterial etiology of community acquired pneumonia. *Tropical Biomedicine*. 28: 531–544.

Najdenski, H., Kussovski, V., Vesselinova, A. (2004). Experimental Burkholderia pseudomallei infection of pigs. *Journal of veterinary Medicine. B, Infectious Diseases and Veterinary Public Health*. 51: 225–230.

Nakazawa, T., Yamada, Y., Ishibashi, M. (1987). Characterization of hemolysin in extracellular products of Pseudomonas cepacia. *Journal of Clinical Microbiology*. 25: 195–8.

Nandagopal, B., Sankar, S., Lingesan, K., Appu, K., Sridharan, G., Gopinathan, A. (2012). Application of polymerase chain reaction to detect Burkholderia pseudomallei and Brucella species in buffy coat from patients with febrile illness among rural and peri-urban population. *Journal of Global Infectious Diseases*. 4: 31–37.

Nandi, T., Ong, C., Singh, A.P., Boddey, J., Atkins, T., Sarkar-Tyson, M., Essex-Lopresti, A.E., Chua, H.H., Pearson, T., Kreisberg, J.F., Nilsson, C., Ariyaratne, P., Ronning, C., Losada, L., Ruan, Y., Sung, W.-K., Woods, D., Titball, R.W., Beacham, I., Peak, I., Keim, P., Nierman, W.C., Tan, P. (2010). A genomic survey of positive selection in Burkholderia pseudomallei provides insights into the evolution of accidental virulence. *PLoS Pathogens*. 6: e1000845.

Nathan, S., Chieng, S., Su, Y. (2006). Cloning and Expression of a Burkholderia pseudomallei Putative Peptidase M23B. *Malaysian Journal for Biochemistry and Molecular Biology*. 14: 33–37.

Nathan, S., Leong, Y.T., Sa'ariwijaya, M.S.F. (2005). Burkholderia pseudomallei Secretes Metallo- and Serine Proteases in Culture. *Malaysian Journal for Biochemistry and Molecular Biology*. 11: 13–19.

Nathan, S.A., Puthucheary, S.D. (2005). An electronmicroscopic study of the interaction of Burkholderia pseudomallei and human macrophages. *The Malaysian Journal of Pathology*. 27: 3–7.

Nelson, M., Dean, R.E., Salguero, F.J., Taylor, C., Pearce, P.C., Simpson, A.J.H., Lever, M.S. (2011). Development of an acute model of inhalational melioidosis in the common marmoset (Callithrix jacchus). *International Journal of Experimental Pathology*. 92: 428–435.

Neubauer, H., Sprague, L.D., Joseph, M., Tomaso, H., Dahouk, S. Al, Witte, A., Kinne, J., Hensel, A., Wernery, R., Wernery, U., Scholz, H.C. (2007). Development and Clinical Evaluation of A PCR Assay Targeting the Metalloprotease Gene (mprA) of B. pseudomallei. *Zoonosis and Public Health*. 54: 44–50.

Nicholas, J.V., Deshazer, D. (2008). Melioidosis in medical aspects of biological warfare, 1st: ed.

Nigg, C., Heckly, R.J., Colling, M. (1955). Toxin produced by Malleomyces pseudomallei. *Proceedings of the Society for Experimental Biology and Medicine (New York, N.Y.)*. 89: 17–20.

Novak, R.T., Glass, M.B., Gee, J.E., Gal, D., Mayo, M.J., Currie, B.J., Wilkins, P.P. (2006). Development and evaluation of a real-time PCR assay targeting the type III secretion system of Burkholderia pseudomallei. *Journal of Clinical Microbiology*. 44: 85–90.

O'Quinn, A.L., E. M. Wiegand, Jeddeloh, J.A. (2001). Burkholderia pseudomallei kills the nematode Caenorhabditis elegans using an endotoxin-mediated paralysis. *Cellular Microbiology*. 3: 381–398.

Peacock, S.J., Limmathurotsakul, D., Lubell, Y., Koh, G.C.K.W., White, L.J., Day,

N.P.J., Titball, R.W. (2012). Melioidosis vaccines: A systematic review and appraisal of the potential to exploit biodefense vaccines for public health purposes. *PLoS Neglected Tropical Diseases*. 6: e1488.

Perry, M.B., MacLean, L.L., Schollaardt, T., Bryan, L.E., Ho, M. (1995). Structural characterization of the lipopolysaccharide O antigens of Burkholderia pseudomallei. *Infection and Immunity*. 63: 3348–3352.

Piggott, J.A., Hochholzer, L. (1970). Human melioidosis. A histopathologic study of acute and chronic melioidosis. *Archives of Pathology*. 90: 101–111.

Pilatz, S., Breitbach, K., Hein, N., Fehlhaber, B., Schulze, J., Brenneke, B., Eberl, L., Steinmetz, I. (2006). Identification of Burkholderia pseudomallei genes required for the intracellular life cycle and in vivo virulence. *Infection and Immunity*. 74: 3576–3586.

Pitt, T.L., Trakulsomboon, S., Dance, D.A.B. (2000). Molecular phylogeny of Burkholderia pseudomallei. *Acta Tropica*. 74: 181–185.

Podin, Y., Sarovich, D.S., Price, E.P., Kaestli, M., Mayo, M., Hii, K., Ngian, H., Wong, S., Wong, I., Wong, J.J., Mohan, A., Ooi, M., Fam, T., Tuanyok, A., Keim, P., Giffard, P.M., Currie, B.J. (2014). Burkholderia pseudomallei isolates from Sarawak, Malaysian Borneo, are predominantly susceptible to aminoglycosides and macrolides. *Antimicrobial Agents and Chemotherapy*. 58: 162–166.

Pongsunk, S., Thirawattanasuk, N Piyasangthong, N., Ekpo, P. (1999). Rapid identification of Burkholderia pseudomallei in blood cultures by a monoclonal antibody assay. *Journal of Clinical Microbiology*. 37: 3662–3667.

Prakash, A., Thavaselvam, D., Kumar, A., Kumar, A., Arora, S., Tiwari, S., Barua, A., Sathyaseelan, K. (2014). Isolation, identification and characterization of Burkholderia pseudomallei from soil of coastal region of India. *SpringerPlus*. 3: 438.

Price, E.P., Dale, J.L., Cook, J.M., Sarovich, D.S., Seymour, M.L., Ginther, J.L., Kaufman, E.L., Beckstrom-Sternberg, S.M., Mayo, M., Kaestli, M., Glass, M.B., Gee, J.E., Wuthiekanun, V., Warner, J.M., Baker, A., Foster, J.T., Tan, P., Tuanyok, A., Limmathurotsakul, D., Peacock, S.J., Currie, B.J., Wagner, D.M., Keim, P., Pearson, T. (2012). Development and validation of Burkholderia pseudomallei-specific real-time PCR assays for clinical, environmental or forensic detection applications. *PloS One*. 7: e37723.

Punyagupta, S. (1989). Melioidosis: Review of 686 cases and presentation of a new clinical classification. In: Punyagupta, S., Sirisanthana, T., Stapatayavong, B. (Eds.), Melioidosis: Bangkok.

Radu, S., Ling, O.W., Srimontree, S., Lulitanond, A., Hin, W.F., Yuherman, Lihan, S., Rusul, G., Mutalib, A.R. (2000). Characterization of Burkholderia pseudomallei isolated in Thailand and Malaysia. *Diagnostic Microbiology and Infectious Disease*. 38: 141–5.

Rainbow, L., Wilkinson, M.C., Sargent, P.J., Hart, C.A., Winstanley, C. (2004).

Identification and expression of a Burkholderia pseudomallei collagenase in Escherichia coli. *Current Microbiology*. 48: 300–304.

Rattanathongkom, A., Sermswan, R.W., Wongratanacheewin, S. (1997). Detection of Burkholderia pseudomallei in blood samples using polymerase chain reaction. *Molecular and Cellular Probes*. 11: 25–31.

Raveneau, J., Geoffroy, C., Beretti, J.L., Gaillard, J.L., Alouf, J.E., Berche, P. (1992). Reduced virulence of a Listeria monocytogenes phospholipase-deficient mutant obtained by transposon insertion into the zinc metalloprotease gene. *Infection and Immunity*. 60: 916–921.

Raynaud, C., Guilhot, C., Rauzier, J., Bordat, Y., Pelicic, V., Manganelli, R., Smith, I., Gicquel, B., Jackson, M. (2002). Phospholipases C are involved in the virulence of Mycobacterium tuberculosis. *Molecular Microbiology*. 45: 203–217.

Razak, N., Ismail, G. (1982). Interaction of Human Polymorhonuclear Leukocytes with Pseudomonas pseudomallei. *Journal of General and Applied Microbiology*. 28: 509–518.

Reganzerowski, A., Hoppe, I., Brenneke, B., Rohde, M., Kreft, A., Ha, S. (1999). Characterization of a murine model of melioidosis : comparison of different strains of mice. *Infection and Immunity*. 67: 2891–2900.

Rietschel, E.T., Kirikae, T., Schade, F.U., Mamat, U., Schmidt, G., Loppnow, H., Ulmer, A.J., Zähringer, U., Seydel, U., Di Padova, F. (1994). Bacterial endotoxin: molecular relationships of structure to activity and function. *Federation of American Societies for Experimental Biology*. 8: 217–225.

Roux, E., Yersin, A. (1889). Contribution a l'etude de la diphtherie.

Sam, I.-C., Puthucheary, S.D. (2006). Melioidosis in children from Kuala Lumpur, Malaysia. *Annals of Tropical Paediatrics*. 26: 219–224.

Sam, I.-C., Puthucheary, S.D. (2007). Melioidosis and rainfall in Kuala Lumpur, Malaysia. *The Journal of Infection*. 54: 519–520.

Samosornsuk, N., Lulitanond, A., Saenla, N., Anuntagool, N Wongratanacheewin, S Sirisinha, S. (1999). Short report: evaluation of a monoclonal antibody-based latex agglutination test for rapid diagnosis of septicemic melioidosis. *The American Journal of Tropical Medicine and Hygine*. 61: 735–737.

Sanford, J. (1995). Pseudomonas Species (Including Melioidosis and Glanders), *Principles and Practice of Infectious Diseases*: Principles and Practice of Infectious Diseases, Churchill Livingstone, New York.

Sapian, M., Khairi, M.T., How, S.H., Rajalingam, R., Sahhir, K., Norazah, A., Khebir, V., Jamalludin, A.R. (2012). Outbreak of melioidosis and leptospirosis co-infection. *Medical Journal of Malaysia*. 67: 293–297.

Saravu, K., Mukhopadhyay, C., Vishwanath, S., Valsalan, R., Docherla, M., Bairy, I. (2010). Melioidosis in southern India: Epidemiological and clinical profile. *Southeast Asian Journal of Tropical Medicine and Public Health*.

41: 401–409.

Schmitt, C.K., Meysick, K.C., O'Brien, A.D. (1999). Bacterial toxins: friends or foes? *Emerging Infectious Diseases*. 5: 224–234.

Schully, K.L., Bell, M.G., Ward, J.M., Keane-Myers, A.M. (2014). Oropharyngeal aspiration of Burkholderia mallei and Burkholderia pseudomallei in BALB/c Mice. *PloS One*. 9: e115066.

Sexton, M.M., Jones, A.L., Chaowagul, W., Woods, D.E. (1994). Purification and characterization of a protease from Pseudomonas pseudomallei. *Canadian Journal of Microbiology*. 40: 903–910.

Shah, K., Mody, K., Keshri, J., Jha, B. (2010). Purification and characterization of a solvent, detergent and oxidizing agent tolerant protease from Bacillus cereus isolated from the Gulf of Khambhat. *Journal of Molecular Catalysis B: Enzymatic*. 67: 85–91.

Sim, S.H., Yu, Y., Lin, C.H., Karuturi, R.K.M., Wuthiekanun, V., Tuanyok, A., Chua, H.H., Ong, C., Paramalingam, S.S., Tan, G., Tang, L., Lau, G., Ooi, E.E., Woods, D., Feil, E., Peacock, S.J., Tan, P. (2008). The core and accessory genomes of Burkholderia pseudomallei: implications for human melioidosis. *PLoS Pathogens*. 4: e1000178.

Sirisinha, S., Anuntagool, N., Dharakul, T., Ekpo, P., Wongratanacheewin, S., Naigowit, P., Petchclai, B., Thamlikitkul, V., Suputtamongkol, Y. (2000). Recent developments in laboratory diagnosis of melioidosis. *Acta Tropica*. 74: 235–245.

Smith, C.J., Allen, J.C., Embi, M.N., Othman, O., Razak, N., Ismail, G. (1987). Human melioidosis: an emerging medical problem. *Mircen Journal of Applied Microbiology and Biotechnology*. 3: 343–366.

Smith, G.S., Walter, G.L., Walker, R.M. (2013). Haschek and Rousseaux's Handbook of Toxicologic Pathology, *Academic press*: Academic press.

Smith, M.D., Angus, B.J., Wuthiekanun, V., White, N.J. (1997). Arabinose assimilation defines a nonvirulent biotype of Burkholderia pseudomallei. *Infection and Immunity*. 65: 4319–4321.

Smith-Vaughan, H.C., Gal, D., Lawrie, P.M., Winstanley, C., Sriprakash, K.S., Currie, B.J. (2003). Ubiquity of putative type III secretion genes among clinical and environmental Burkholderia pseudomallei isolates in Northern Australia. *Journal of Clinical Microbiology*. 41: 883–885.

Soffler, C., Bosco-Lauth, A.M., Aboellail, T.A., Marolf, A.J., Bowen, R.A. (2012). Development and characterization of a caprine aerosol infection model of melioidosis. *PloS One*. 7: e43207.

Soffler, C., Bosco-lauth, A.M., Aboellail, T.A., Marolf, A.J., Bowen, R.A. (2014). Pathogenesis of percutaneous infection of goats with Burkholderia pseudomallei: clinical, pathologic, and immunological responses in chronic melioidosis. *International Journal of Experimental Pathology*. 95Soffler,: 101–119.

Songer, J.G. (1997). Bacterial phospholipases and their role in virulence. *Trends in Microbiology*. 5: 156–161.

Sonthayanon, P., Krasao, P., Wuthiekanun, V., Panyim, S., Tungpradabkul, S. (2002). A simple method to detect and differentiate Burkholderia pseudomallei and Burkholderia thailandensis using specific flagellin gene primers. *Molecular and Cellular Probes*. 16: 217–222.

Spinosa, M.R., Progida, C., Talà, A., Cogli, L., Alifano, P., Bucci, C. (2007). The Neisseria meningitidis capsule is important for intracellular survival in human cells. *Infection and Immunity*. 75: 3594–3603.

Sprague, L.D., Neubauer, H. (2004). Melioidosis in animals: A review on epizootiology, diagnosis and clinical presentation. *Journal of veterinary medicine. B, Infectious diseases and veterinary public health*. 51: 305–320.

Stanton, A.T., Fletcher, W. (1932). Melioidosis. Studies from the Institute of Medical Research, Federated Malay States.

Steinmetz, I., Reganzerowski, A., Brenneke, B., Haussler, S., Simpson, A., White, N.J. (1999). Rapid identification of Burkholderia pseudomallei by latex agglutination based on an exopolysaccharide-specific monoclonal antibody. *Journal of Clinical Microbiology*. 37: 225–228.

Steinmetz, I., Rohde, M., Brenneke, B. (1995). Purification and characterization of an exopolysaccharide of Burkholderia (Pseudomonas) pseudomallei. *Infection and Immunity*. 63: 3959–3965.

Stevens, M.P., Haque, A., Atkins, T., Hill, J., Wood, M.W., Easton, A., Nelson, M., Underwood-Fowler, C., Titball, R.W., Bancroft, G.J., Galyov, E.E. (2004). Attenuated virulence and protective efficacy of a Burkholderia pseudomallei bsa type III secretion mutant in murine models of melioidosis. *Microbiology*. 150: 2669–2676.

Stevens, M.P., Wood, M.W., Taylor, L.A., Monaghan, P., Hawes, P., Jones, P.W., Wallis, T.S., Galyov, E.E. (2002). An Inv/Mxi-Spa-like type III protein secretion system in Burkholderia pseudomallei modulates intracellular behaviour of the pathogen. *Molecular Microbiology*. 46: 649–659.

Suppiah, J., Thimma, J.S., Cheah, S.H., Vadivelu, J. (2010). Development and evaluation of polymerase chain reaction assay to detect Burkholderia genus and to differentiate the species in clinical specimens. *FEMS Microbiology Letters*. 306: 9–14.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*. 28: 2731–2739.

Titball, R.W. (1993). Bacterial Phospholipases C. *Microbiological Reviews*. 57: 347–366.

Titball, R.W., Russell, P., Cuccui, J., Easton, A., Haque, A., Atkins, T., Sarkar-Tyson, M., Harley, V., Wren, B., Bancroft, G.J. (2008). Burkholderia

pseudomallei: animal models of infection. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 102 Suppl: S111–S116.

Tkachenko, G.A., Antonov, V.A., Zamaraev, V.S., Iliukhin, V.I. (2003). Identification of the causative agents of glanders and melioidosis by polymerase chain reaction. *Molekuliarnaia Genetika, Mikrobiologiia i Virusologiia*. 3: 18–22.

Tomaso, H., Pitt, T.L., Landt, O., Al Dahouk, S., Scholz, H.C., Reisinger, E.C., Sprague, L.D., Rathmann, I., Neubauer, H. (2005). Rapid presumptive identification of Burkholderia pseudomallei with real-time PCR assays using fluorescent hybridization probes. *Molecular and Cellular Probes*. 19: 9–20.

Tomaso, H., Scholz, H.C., Al Dahouk, S., Pitt, T.L., Treu, T.M., Neubauer, H. (2004). Development of 5' nuclease real-time PCR assays for the rapid identification of the Burkholderia mallei/Burkholderia pseudomallei complex. *Diagnostic Molecular Pathology*. 13: 247–253.

Tomlinson, L., Boone, L.I., Ramaiah, L., Penraat, K.A., von Beust, B.R., Ameri, M., Poitout-Belissent, F.M., Weingand, K., Workman, H.C., Aulbach, A.D., Meyer, D.J., Brown, D.E., Macneill, A.L., Bolliger, A.P., Bounous, D.I. (2013). Best practices for veterinary toxicologic clinical pathology, with emphasis on the pharmaceutical and biotechnology industries. *Veterinary Clinical Pathology*. 42: 252–269.

Tonpitak, W., Sornklien, C., Chanawit, M., Pavasutthipaisit, S., Wuthiekanun, V., Hantrakun, V., Amornchai, P., Thaipadungpanit, J., Day, N.P.J., Yingst, S., Others (2014). Fatal Melioidosis in Goats in Bangkok, Thailand. *The American Journal of Tropical Medicine and Hygiene*. 91: 14–115.

Trakulsomboon, S., Dance, D.A., Smith, M.D., White, N.J., Pitt, J.L. (1997). Ribotype differences between clinical and environmental isolates of B. pseudomallei. *Journal of Medical Microbiology*. 46: 565–570.

Trakulsomboon, S., Vuddhakul, V., Tharavichitkul, P., Na-Gnam, N., Suputtamongkol, Y Thamlikitkul, V. (1999a). Epidermiology of arabinose assimilation in Burkholderia pseudomallei isolates from patients and soil in Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health*. 30: 756–759.

Trakulsomboon, S., Vuddhakul, V., Tharavichitkul, P., Na-Gnam, N., Suputtamongkol, Y., Thamlikitkul, V. (1999b). Epidemiology of arabinose assimilation in Burkholderia pseudomallei isolated from patients and soil in Thailand. *The Southeast Asian Journal of Tropical Medicine and Public Health*. 30: 756–759.

Trung, T.T., Hetzer, A., Göhler, A., Topfstedt, E., Wuthiekanun, V., Limmathurotsakul, D., Peacock, S.J., Steinmetz, I. (2011). Highly sensitive direct detection and quantification of Burkholderia pseudomallei bacteria in environmental soil samples by using real-time PCR. *Applied and Environmental Microbiology*. 77: 6486–6494.

Tumapa, S., Holden, M.T.G., Vesaratchavest, M., Wuthiekanun, V., Limmathurotsakul, D., Chierakul, W., Feil, E.J., Currie, B.J., Day, N.P.J., Nierman, W.C., Peacock, S.J. (2008). Burkholderia pseudomallei genome plasticity associated with genomic island variation. *BMC Genomics*. 9: 190.

Tyler, S.D., Strathdee, C.A., Rozee, K.R. (1995). Oligonucleotide Primers Designed To Differentiate Pathogenic Pseudomonads on the Basis of the Sequencing of Genes Coding for 16S-23S rRNA Internal Transcribed Spacers. *Clinical and Diagnostic Laboratory Immunology*. 2: 448–453.

Ulett, G.C., Currie, B.J., Clair, T.W., Mayo, M., Ketheesan, N., Labrooy, J., Gal, D., Norton, R., Ashhurst, C., Barnes, J., Warner, J., Hirst, R.G. (2001). Burkholderia pseudomallei virulence: definition, stability and association with clonality. *Microbes and Infection*. 3: 621–631.

Ulett, G.C., Ketheesan, N., Hirst, R.G. (2000). Cytokine gene expression in innately susceptible BALB/c mice and relatively resistant C57BL/6 mice during infection with virulent Burkholderia pseudomallei. *Infection and Immunity*. 68: 2034–2042.

Ulrich, R.L. (2004). Role of quorum sensing in the pathogenicity of Burkholderia pseudomallei. *Journal of Medical Microbiology*. 53: 1053–1064.

Vadivelu, J., Puthucheary, S.D., Mifsud, A., Drasar, B.S., Dance, D.A., Pitt, T.I. (1997). Ribotyping and DNA macrorestriction analysis of isolates of Burkholderia pseudomallei from cases of melioidosis in Malaysia. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 91: 358–360.

Van der Lugt, J.J., Henton, M.M. (1995). Melioidosis in a Goat. *Journal of the South African Veterinary Association*. 66: 71–73.

Van Zandt, K.E., Tuanyok, A., Keim, P.S., Warren, R.L., Gelhaus, H.C. (2012). An objective approach for Burkholderia pseudomallei strain selection as challenge material for medical countermeasures efficacy testing. *Frontiers in Cellular and Infection Microbiology*. 2: 120.

Vandeputte-Rutten, L., Gros, P. (2002). Novel proteases: common themes and surprising features. *Current Opinion in Structural Biology*. 12: 704–708.

Velianov, D., Naĭdenski, K. (1993). Virulence and susceptibility to phagocytosis of Pseudomonas pseudomallei R- and S-forms for ground squirrels (Citellus citellus L.). *Acta Microbiologica Bulgarica*. 30: 11–16.

Veljanov, D., Vesselinova, A., Nikolova, S., Najdenski, H., Kussovski, V., Markova, N. (1996). Experimental melioidosis in inbred mouse strains. *Zentralblatt für Bakteriologie*. 283: 351–359.

Vellasamy, K.M., Vasu, C., Puthucheary, S.D., Vadivelu, J. (2009). Comparative analysis of extracellular enzymes and virulence exhibited by Burkholderia pseudomallei from different sources. *Microbial Pathogenesis*. 47: 111–117.

Vongphayloth, K., Rattanavong, S., Moore, C.E., Phetsouvanh, R., Wuthiekanun, V., Sengdouangphachanh, A., Phouminh, P., Newton, P.N., Buisson, Y.

(2012). Burkholderia pseudomallei detection in surface water in southern Laos using Moore's swabs. *The American Journal of Tropical Medicine and Hygiene*. 86: 872–877.

Vuddhakul, V., Tharavichitkul, P., Na-Ngam, N., Jitsurong, S., Kunthawa, B., Noimay, P., Noimay, P., Binla, A., Thamlikitkul, V. (1999). Epidemiology of Burkholderia pseudomallei in Thailand. *The American Journal of Tropical Medicine and Hygine*. 60: 458–461.

Wajanarogana, S., Sonthayanon, P., Simpson, A.J.H., Tungpradabkul, S., Panyini, S. (1999). Stable marker on flagellin gene sequences to arabinose non-assimilating pathogenic Burkholderia pseudomallei. *Microbiology and Immunology*. 43: 995–1001.

Walia, K., Ghosh, S., Singh, H., Nair, G.B., Ghosh, A., Sahni, G., Vohra, H., Ganguly, N.K. (1999). Purification and Characterization of Novel Toxin Produced by Vibrio cholerae O1. *Infection and Immunity*. 67: 5215–5222.

Walsh, A.L., Smith, M.D., Wuthiekanun, V., Suputtamongkol, Y., Chaowagul, W., Dance, D.A., Angus, B., White, N.J. (1995). Prognostic significance of quantitative bacteremia in septicemic melioidosis. *Clinical Infectious Diseases*. 21: 1498–500.

Walsh, A.L., Wuthiekanun, V. (1996). The laboratory diagnosis of melioidosis. *British Journal of Biomedical Science*. 53: 249–53.

Wand, M.E., Müller, C.M., Titball, R.W., Michell, S.L. (2011). Macrophage and Galleria mellonella infection models reflect the virulence of naturally occurring isolates of B. pseudomallei, B. thailandensis and B. oklahomensis. *BMC Microbiology*. 11: 1–11.

Warawa, J., Woods, D.E. (2002). Melioidosis vaccines. *Expert Review of Vaccines*. 1: 477–482.

Warrasuth, N., Thapanagulsak, P., Anun, S., Joraka, W., Wongratanacheewin, S., Day, N.P.J., Peacock, S.J. (2012). Melioidosis in animals, Thailand 2006–2010. *Emerging Infectious Diseases*. 18: 325–327.

Welch, J., McClelland, M. (1990). Fingerprinting genomes using PCR with Arbitary Primers. *Nucleic Acids Reviews*. 18: 7213–7218.

White, N.J. (2003). Melioidosis. *Lancet*. 361: 1717–1722.

Whitmore, A., Krishnaswami, C. (1912). An account of the discovery of a hitherto undescribed infective disease occurring among the population of Rangoon. *Indian Medical Gazette*. 47: 262–267.

Wikraiphat, C., Charoensap, J., Utaisincharoen, P., Wongratanacheewin, S., Taweechaisupapong, S., Woods, D.E., Bolscher, J.G.M., Sirisinha, S. (2009). Comparative in vivo and in vitro analyses of putative virulence factors of Burkholderia pseudomallei using lipopolysaccharide, capsule and flagellin mutants. *FEMS Immunology and Medical Microbiology*. 56: 253–259.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A., Tingly, S.V. (1990). DNA polymorphisms amplified by arbitary primers are useful as genetic markers. *Nucleic Acids Reviews*. 18: 6531–6535.

Wilson, J.W. (2002). Mechanisms of bacterial pathogenicity. *Postgraduate Medical Journal*. 78: 216–224.

Winstanley, C., Hales, B.A., Corkill, J.E., Gallagher, M.J., Hart, C.A. (1998). Flagellin gene variation between clinical and environmental isolates of Burkholderia pseudomallei contrasts with the invariance among clinical isolates. *Journal of Medical Microbiology*. 47: 689–694.

Winstanley, C., Hales, B.A., Hart, C.A. (1999). Evidence for the presence in Burkholderia pseudomallei of a type III secretion system-associated gene cluster. *Journal of Medical Microbiology*. 48: 649–656.

Winstanley, C., Hart, C.A. (2000). Presence of type III secretion genes in Burkholderia pseudomallei correlates with ara + phenotypes. *Journal of Clinical Microbiology*. 38: 883–885.

Wong, K.T.T., Puthucheary, S.D.D., Vadivelu, J. (1995). The histopathology of human melioidosis. *Histopathology*. 26: 51–55.

Wongratanacheewin, S., Kespichayawattana, W., Intachote, P., Pichyangkul, S., Sermswan, R.W., Krieg, A.M., Sirisinha, S. (2004). Immunostimulatory CpG oligodeoxynucleotide confers protection in a murine model of infection with Burkholderia pseudomallei. *Infection and Immunity*. 72: 4494–4502.

Woods, D., DeShazer, D., Moore, R., Brett, P., Burtnick, M., Reckseidler, S., Senkiw, M. (1999). Current studies on the pathogenesis of melioidosis. *Microbes and Infection*. 1: 157–162.

Wuthiekanun, V., Amornchai, P., Saiprom, N., Chantratita, N., Chierakul, W., Koh, G.C.K.W., Chaowagul, W., Day, N.P.J., Limmathurotsakul, D., Peacock, S.J. (2011). Survey of antimicrobial resistance in clinical Burkholderia pseudomallei isolates over two decades in Northeast Thailand. *Antimicrobial Agents and Chemotherapy*. 55: 5388–5391.

Wuthiekanun, V., Desakorn, V., Wongsuvan, G., Amornchai, P., Cheng, A.C., Maharjan, B Limmathurotsakul, D Chierakul, W., White, N.J., Day, N.P.J., Peacock, S.J. (2005). Rapid immunofluorescence microscopy for diagnosis of meliodosis. *Clinical and Diagnostic Laboratory Immunology*. 12: 555–556.

Yabuuchi, E., Kosako, Y., Oyaizu, H., Yano, I., Hotta, H., Hashimoto, Y., Ezaki, T., Arakawa, M. (1992). Proposal of Burkholderia gen. nov. and transfer of seven species of the genus Pseudomonas homology group II to the new genus, with the type species Burkholderia cepacia (Palleroni and Holmes 1981) comb. nov. *Microbiology and Immunology*. 36: 1251–1275.

Yap, E.P.H., Ang, S.M., Seah, S.G.K., Phang, S.M. (2002). Homogeneous assays for the rapid PCR detection of Burkholderia pseudomallei 16S rRNA gene on a real- time fluorometric capillary thermocycler. In Rapid Cycle Real

Time PCR - Methods and Application, in: *Microbiology and Food Analysis*Microbiology and Food Analysis, (Berlin, Springer), pp. 59–67.

Yeager, J.J., Facemire, P., Dabisch, P.A., Robinson, C.G., Nyakiti, D., Beck, K., Baker, R., Pitt, M.L.M. (2012). Natural history of inhalation melioidosis in rhesus macaques (Macaca mulatta) and African green monkeys (Chlorocebus aethiops). *Infection and Immunity*. 80: 3332–3340.

Yu, Y., Kim, H.S., Chua, H.H., Lin, C.H., Sim, S.H., Lin, D., Derr, A., Engels, R., DeShazer, D., Birren, B., Nierman, W.C., Tan, P. (2006). Genomic patterns of pathogen evolution revealed by comparison of Burkholderia pseudomallei, the causative agent of melioidosis, to avirulent Burkholderia thailandensis. *BMC Microbiology*. 6: 46.

Zhang, B., Wear, D.J., Kim, H.S., Weina, P., Stojadinovic, A., Izadjoo, M. (2012). Development of hydrolysis probe-based real-time PCR for identification of virulent gene targets of Burkholderia pseudomallei and B. mallei: A retrospective study on archival cases of service members with melioidosis and glanders. *Military Medicine*. 177: 216–221.

Zhang, Z., Schwartz, S., Wagner, L., Miller, W. (2000). A greedy algorithm for aligning DNA sequences. *Journal of Computational Biology*. 7: 203–214.