

出國報告（出國類別：參加會議）

參加 2012 年歐洲類鼻疽網路會議

出國報告

The report of attending the 2012 International
Conference on European Melioidosis Network
Meeting

服務機關：國立屏東科技大學

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派赴國家：阿姆斯特丹 荷蘭

出國期間：民國 101 年 2 月 18 日至 2 月 25 日

摘要

本次會議為 2012 年歐洲類鼻疽網路會議 (European Melioidosis Network Meeting) 在荷蘭首都阿姆斯特丹 (Amsterdam) 舉行，此次出國由本人和博士班學生魯懿萍小姐和中台科技大學潘銘正教授等三人，因先來到荷蘭，離會議期間尚有幾天，故藉此機會以百聞不如一見的心情先觀察荷蘭在教育和文化的成就，以增廣見聞，之後再參加於 2012 年 2 月 24 日在荷蘭首都阿姆斯特丹 (Amsterdam) 舉行的會議。本次會議共分四個主題和壁報參展，第一個主題是有關類鼻疽的分子生物學和致病機制：由新加坡的 Patrick Tan、英國的 Joann Prior 和泰國的 Ganjana Lertmemongkolchai 三位醫師報告。第二個主題是有關類鼻疽感染的危險因子、傳染途徑和患有糖尿病人的角色：由泰國 Direk Limmathurotsakul、英國的 Gavin Koh 和新加坡的 Yunn-Hwen Gan 三位醫師報告。第三個主題是有關類鼻疽的預防新知：由英國的 Richard Tittball、美國的 Herbert Schweizer 和美國的 Steven Dow 三位醫師報告。第四個主題是有關類鼻疽的綜說和頒發從事類鼻疽研究的年青人獎：由德國的 Ivo Steinmetz 和澳洲的 Bart Currie 二位醫師報告，而年青人獎由德國的 Kathrin Matschinski 獲得並演講。壁報部份共有 35 篇，其中由本校發表的壁報有 2 篇(如附件)。從參加這次會議覺得類鼻疽是一種非常重要的人畜共通傳染病，在國外大部分是由醫師(人醫)在主導本病的相關研究，從會議的內容可見，國外的研究除基礎醫學做得相當深入外，在防控此病上也相當廣泛的在探討，如何有效的來防控此病的發生，以增進人類的健康。反觀我國對本病的研究資源較缺乏，在獸醫方面目前只有本校和屏東縣家畜疾病防治所積極在進行，建議政府應重視此病的危害，加強對本病的研究，以保障國人的健康。藉由本次會議，和國外學者專家相互切磋，增廣自己的實務經驗與見聞。並在類鼻疽網路會議舞台上展現台灣在類鼻疽領域之研究成果與貢獻，最後覺得此行收獲頗多，將來將此收獲貢獻給本校和國家。

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壹、目的

今年二月，參加 2012 年歐洲類鼻疽網路會議 (European Melioidosis Network Meeting)，本人參加聽取各國學者專家對類鼻疽最新的相關發展，獲益良多。以利爾後對類鼻疽研究方向的依據。也藉此會議展現台灣在類鼻疽領域之研究成果與貢獻。

貳、過程

2 月 18 日：

早上 8:40 在桃園國際機場搭乘長榮航空 BR75 班機經曼谷直飛荷蘭阿姆斯特丹，於 2 月 18 日當地時間晚上約 8 點抵達阿姆斯特丹 Schiphol 機場。

2 月 19-23 日：

我們一行三人因提前到荷蘭，距開會時間尚有幾天，藉此機會一方面觀察荷蘭這個國家在教育上、社會上、經濟上、文化上等等的建設水準外，另一方面更積極收集與教學上有相關的資料，如參訪烏特勒之 (Oudegracht) 大學時，將學校的硬體建築和教學上設備的特色記錄並照相下來，將此資料準備回國後，製作教學幻燈片，並利用上課時間放映給學生觀賞，一方面解說荷蘭在文化、經濟、建設和各方面的優點，另一方面與學生互相討論，增廣見聞，達成教學相長的目的。

2 月 24 日：

參加會議，本次會議為 2012 年歐洲類鼻疽網路會議 (European Melioidosis Network Meeting)，於 2012 年 2 月 24 日在荷蘭首都阿姆斯特丹 (Amsterdam) 舉行，當天來自全世界從事類鼻疽研究的重要學者專家幾乎全部到齊。此次會議於 2 月 24 日早上開始報到，之後隨即舉行研討會，第一個主題是有關類鼻疽的分子生物學和致病機制：由新加坡的 Patrick Tan 醫師演講類鼻疽菌基因體的新發現，殺草劑可殺死此菌，但對動物無害、英國的 Joann Prior 醫師

演講類鼻疽菌莢膜多醣體的構造和功能 and 泰國的 Ganjana Lertmemongkolchai 醫師演講人類嗜中性球對類鼻疽菌的反應，以共軛焦顯微鏡觀察此現象，發現嗜中性球吞噬類鼻疽菌扮演重要的角色。第二個主題是有關類鼻疽感染的危險因子、傳染途徑和患有糖尿病病人的角色：由泰國 Direk Limmathurotsakul 醫師演講類鼻疽傳染的途徑，以接觸、食入和吸入為主，並利用病例對照研究分析此病、英國的 Gavin Koh 醫師演講患糖尿病感染此菌的影響和 glibenclamide 在本菌的致病過程中的影響，發現糖尿病患者是感染此病最主要的危險因子和新加坡的 Yunn-Hwen Gan 醫師演講在糖尿病患者有那些因子對感染本病會增加其敏感性。中午休息時間有壁報展示和討論，這次本人和本校博士生提供 2 篇壁報，其題目分別為 Antimicrobial susceptibility in goat isolates of *Burkholderia pseudomallei* and molecular database construction of *Burkholderia pseudomallei* isolated from goat using phylogenetic analysis and fingerprinting markers 和 Characterization of *Burkholderia pseudomallei* isolates from goat in Taiwan. 本人皆列為通訊作者，主要以台灣在羊隻發生的病例和相關研究發表在壁報上，此為本台灣在羊隻發生類鼻疽的第一篇報告，具有指標意義。並一起和與會領域相同的學者專家有深入的切磋的機會，許多學者專家也一致肯定和讚許壁報內容的成果，並鼓勵發表在國際雜誌上。第三個主題是有關類鼻疽的預防新知：由英國的 Richard Tittball 醫師演講最近對本病有關疫苗的研究，發現疫苗和免疫促進劑併用，其效果會更好、美國的 Herbert Schweizer 醫師演講本菌的抗藥性和治療的策略與最新治療的策略和美國的 Steven Dow 醫師演講應用 TLR-based 免疫治療來預防本菌的感染，發現以口服免疫可以降低慢性類鼻疽的感染。第四個主題是有關類鼻疽的綜說和頒發從事類鼻疽研究的年青人獎：由德國的 Ivo Steinmetz 醫師演講本菌在細胞內的生活使和澳洲的 Bart Currie 醫師演講應用抗生物質治療清除本菌的時間，內容充實，相

當有實用性，可做為今後清除本菌參考的依據，而年青人獎由德國的 Kathrin Matschinski 獲得並演講在老鼠吞噬細胞的 caspases 因子在刺激活化本菌的感染扮演的角色。結束前由泰國 Direk Limmathurotsakul 醫師介紹第七屆世界類鼻疽會議於 2013 年 9 月 18-20 日在泰國曼谷舉辦的相關事項，期盼大家來參加。

2 月 25 日：

在阿姆斯特丹市區參訪旅遊，晚上 21:40 從阿姆斯特丹 Schiphol 機場搭乘長榮航空 BR76 班機經曼谷直飛，於 26 日凌晨返抵桃園國際機場。

參、心得：

- 一、本人以百聞不如一見的心情觀察荷蘭這個國家在教育上、社會上、經濟上、文化上等等的建設水準外，另一方面更積極收集與教學上有相關的資料，如參訪烏特勒之（Oudegracht）大學時，將學校的硬體建築和教學設備的特色記錄下來，將此資料準備回國後，製作教學幻燈片，並利用教學時間放映給學生觀賞，一方面解說兩國之間的異同點，另一方面與學生互相討論，增廣見聞，讓將來想到荷蘭求學進修或工作的學生，對荷蘭有更進一步的認識與瞭解，不會對一個學生初次到國外有生疏的感覺，達成教學相長的目的。
- 二、有關類鼻疽的國際學術研討會是一個從事類鼻疽相關研究很重要的學術交流研討會，在重多的病原微生物當中，只對特定的病原微生物舉辦國際的學術研討會是屈指可數，如禽流感、口蹄疫、嚴重急性呼吸道症候群（SARS）、類鼻疽等，由此可見類鼻疽是其中之一，因類鼻疽是一種重要的人畜共通傳染病，其致病原因、致病機制、細菌和細胞之間的相互關係和如何防治是目前對本病的重要課題，每年均在世界各地輪流舉辦，而此次有教師和研究生將研究成果以壁報展示，和許多與會學者專家一起做學術交流。尤其探討類鼻疽的研究在今年的主題相當不錯，我們希望透過這種國際行學術研討會去開闊視野，為既有的教學和專題研究，注入新的想法和觀念，並奠定良好的專業基礎。
- 三、就個人的心得與啟示而言，本次研討會發表的論文和壁報展示內容相當充實，對本菌的基因體基礎探討和疫苗的開發與藥物的治療均有深入的研究，從參加這次會議覺得類鼻疽是一種非常重要的人畜共通傳染病，在國外大部分是由醫師(人醫)在主導本病的相關研究，從會議的內容看，國外的研究除基礎醫學做得相當深入外，在防控此病上也相當廣泛的在探討，如何有效的來防控此病的發生，以增進人類的健康。反觀我國對本病的研究資源較缺乏，在獸醫方面目前只有本校和屏東縣家畜疾病防治所積極在進行，建議政府應重視此病的危害，加

強對本病的研究，以提昇國人的健康。

四、本次與會學者專家提出許多現今最新的研究技術，並針對防治本菌提供最佳的方法，這些觀點和討論對本人今後的研發有重大的啟示。本次會議雖然只有一天，但由與會的學者專家均是對本病研究最重要的人員，從其發表的論文和壁報本人已受益良多，此外，對本菌今後的研究方向和研究工具的掌握也有非常大的突破，往後對本人的研究具有相當大的助益。從世界各國學者專家投入大筆的經費和人力來探討本菌的相關研究，結合基礎醫學和應用醫學，以期對本菌的防控有所突破，都使本人對此病有更新的瞭解和認識其重要性，參與國際會議可以拓展個人的見解，實感欣慰有此機會瞭解世界各國學者專家相關領域的研究觀點，未來將更積極參與類鼻疽研究相關的國際會議，期望能做為未來研究有更進一步的提昇。

肆、建議事項

- 一、本次參加 2012 年歐洲類鼻疽網路會議，印象深刻。尤其對歐洲和澳洲的學者專家均有精采之作，他們與泰國和新加坡學者專家合作共同探討本病的相關研究，成果頗為豐碩，值得我們學習和更努力的研究。過去，台灣的學術界合作對象也多偏重歐美，但都由我們主動去聯繫，未來可以更擴大合作範圍，朝東南亞和本校有建立姊妹的學校(如泰國、馬來西亞、新加坡、越南等等)聯繫，因本病在東南亞也是常發生的傳染病，藉由以合作方式來探討本病的相關研究，並讓學生更積極的參與本病的相關研究和國際合作，奠定良好的專業基礎和在經驗交流當中開展國際視野，以提昇本校在國際上的能見度。
- 二、從參加這次會議覺得類鼻疽是一種非常重要的人畜共通傳染病，在國外大部分是由醫師(人醫)在主導本病的相關研究，從會議的內容看，國外的研究除基礎醫學做得相當深入外，在防控此病上也相當廣泛的在探討，如何有效的來防控此病的發生，以增進人類的健康。反觀我國對本病的研究資源較缺乏，在獸醫方面目前只有本校和屏東縣家畜疾病防治所積極在進行，建議政府應重視此病的危害，加強對本病的研究，以提昇國人的健康。

伍、附錄

FINAL PROGRAMME EUROPEAN MELIOIDOSIS NETWORK, FEBR. 24, 2012, AMSTERDAM

08.15 Registration and coffee

09.00 Word of Welcome, chair *Joost Wiersinga, University of Amsterdam, the Netherlands*

SESSION I: HOT TOPICS IN MOLECULAR BIOLOGY AND PATHOGENESIS

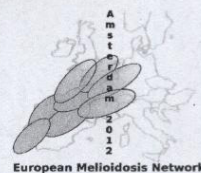
moderator: *Greg Bancroft, London School of Health & Tropical Medicine*

09.00 Key-note lecture: New insights into the genome of *B. pseudomallei*
Patrick Tan, Genome Institute of Singapore

09.30 The structure and function of the *Burkholderia* LPS
Joann Prior, Dstl Porton Down, Salisbury, UK

10.00 Human neutrophil responses to *B. pseudomallei*
Ganjana Lertmemongkolchai, Khon Kaen University, Thailand

10.30 Coffee



SESSION II: RISK FACTORS, ROUTES OF INFECTION AND ROLE OF DIABETES

moderator: *David Dance, Health Protection Agency England*

11.00 Routes of infection in melioidosis
Direk Limmathurotsakul, Mahidol University, Thailand

11.25 The influence of diabetes and glibenclamide on the pathogenesis of melioidosis
Gavin Koh, University of Cambridge, UK

11.50 Factors contributing to increased susceptibility to *B. pseudomallei* infection in diabetic patients
Yunn-Hwen Gan, National University of Singapore

12.15 Round table discussion (until 12.30)

POSTER SESSION, LUNCH PROVIDED

SESSION III: WHAT'S NEW IN MELIOIDOSIS PREVENTION?

moderator: *Sharon Peacock, University of Cambridge*

14.30 An update on vaccine options
Richard Tittball, University of Exeter, UK

14.55 Antibiotic resistance and its impact on therapy and new therapeutic strategies
Herbert Schweizer, Colorado State University, USA

15.20 Use of TLR-based immunotherapeutics for prevention of *Burkholderia* infections
Steven Dow, Colorado State University, USA

15.45 Round table discussion

16.00 Tea

SESSION IV: YOUNG INVESTIGATOR AWARD AND CLOSING SPEAKERS

moderator: *Joost Wiersinga, University of Amsterdam, the Netherlands*

16.30 On the intracellular life cycle of *B. pseudomallei*: an overview
Ivo Steinmetz, University of Greifswald, Germany

17.00 Young Investigator Award: *Bps* infection stimulates activation of caspases in murine macrophages
Kathrin Matschinski, University of Greifswald, Germany

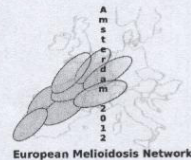
17.15 Is the oral eradication phase of treatment needed for all patients with melioidosis?
Bart Currie, Menzies School of Health Research, Darwin, Australia

17.45 Drinks

POSTER ABSTRACTS AND PRESENTING AUTHORS

1. Immune recognition of polar lipids extracted from *Burkholderia pseudomallei* by caprine dendritic cells and autologous T cells. Torsten M. Eckstein *et al.*, Colorado State University, USA
2. Using *in vivo* imaging to model a *Burkholderia pseudomallei* intranasal infection. Sarah Harding *et al.* Dstl, Porton Down, UK
3. The roles of *mraW* and *tonB* in *B.pseudomallei* virulence. L.E. Marshall *et al.*, Dstl Porton Down, UK
4. Anaerobic respiration in *Burkholderia thailandensis*. Clio Andreae, University of Exeter, UK
5. Host responses to melioidosis and tuberculosis are both dominated by interferon-mediated signalling. Gavin C.K.W. Koh *et al.*, University of Cambridge, UK
6. TraDIS analysis of the *Burkholderia pseudomallei* genome. Madeleine Moule, LSHTM Pathogen Molecular Biology, UK
7. (BALB/c mice can be used to model chronic human infection with Bps if the challenge dose is carefully controlled delivered at a low dose). S Funnell *et al.* Health Protection Agency, UK
8. Autotransported (Type V Secreted) Proteins of *Burkholderia pseudomallei* and Their Role in Biofilm Formation, Serum Resistance and Pathogenesis. N Lazar Adler *et al.*, University of Leicester, Leicester, UK
9. Multistage Vaccine. Olivia L. Champion, University of Exeter, UK
10. Structural Analysis of Capsular Polysaccharides Expressed by *Burkholderia mallei* and *Burkholderia pseudomallei*. Paul J. Brett, University of South Alabama, USA
11. Innate immune responses to *Burkholderia pseudomallei* and *Staphylococcus aureus* in healthy Thai subjects. Narisara Chantratita *et al.*, Mahidol University, Bangkok, Thailand
12. The Bps TA system toxin BPSS0390 causes growth arrest and increases persister cell frequencies: a single amino acid substitution inactivates functionality. Aaron Butt *et al.*, University of Exeter, UK
13. *B. thailandensis* as a high-frequency persister model. Claudia M Mueller *et al.*, University of Exeter, UK
14. An investigation of capsular polysaccharide production in *Burkholderia thailandensis* strain E555. Nicola Senior *et al.*, University of Exeter, UK
15. (Recent outbreak of glanders in Bahrain). Holger Scholz, Bundeswehr Institute of Microbiology, Munich, Germany
16. A nano-glycoconjugate vaccine against melioidosis. Gregory, A. E. University of Exeter, UK
17. Melioidosis and correlates of protection. Thomas R Laws, Dstl Porton Down, UK
18. BPSS1504, a hypothetical T6SS protein, contributes to *in vivo* virulence of *B. pseudomallei* in an Hcp1-independent manner. Katrin Breitbach *et al.*, Friedrich Loeffler Institute of Medical Microbiology, University of Greifswald, Germany
19. *Galleria mellonella* as a high through-put model system to test the efficacy of novel antimicrobials. Rachael J. Thomas *et al.*, College of life and Environmental Sciences, University of Exeter, UK
20. Towards an understanding of the molecular mechanism of *Burkholderia pseudomallei* actin-based motility. Jo Stevens, The Roslin Institute, University of Edinburgh, UK
21. Investigating *Burkholderia* actin tail formation in invertebrate model hosts. Zoë N. Freeman *et al.*, University of Bath, UK
22. Endogenous alpha-2-antiplasmin is protective during melioidosis. Kager LM *et al.*, Center of Experimental and Molecular Medicine (CEMM), Academic Medical Center, Amsterdam, The Netherlands
23. Overexpression of APC is detrimental during experimental melioidosis; Liesbeth M Kager *et al.*, Center of Experimental and Molecular Medicine (CEMM), Academic Medical Center, Amsterdam, The Netherlands

24. Concentrations of *B. pseudomallei* in paddy field soil of Northern Laos. Effects of soil depth, physicochemical properties and seasonality. Manivanh L et al, Institut de la Francophonie pour la Médecine Tropicale, Vientiane, Lao People's Democratic Republic
25. Serine/Threonine protein kinases of *Burkholderia pseudomallei*. Depesh Pankhania et al, University of Leicester Leicester, UK
26. On the role of Toll-like receptor (TLR)-5 during experimental melioidosis. T.A.F. Weehuizen et al, Center of Experimental and Molecular Medicine (CEMM), Academic Medical Center, Amsterdam, The Netherlands
27. Intracellular life cycle of *Burkholderia pseudomallei* in hepatocytes and hepatic defense mechanisms. Imke Schmidt, et al, Friedrich Loeffler Institute of Medical Microbiology, University of Greifswald, Germany
28. Neutrophil extracellular traps (NETs) in the host defence against *Burkholderia pseudomallei*. De Jong HK et al, Center of Experimental and Molecular Medicine (CEMM), Academic Medical Center, Amsterdam, The Netherlands
29. Genomics and Differential Virulence of *Burkholderia pseudomallei* in a BALB/c Mouse Model of Acute Melioidosis. Apichai Tuanyok et al, Center for Microbial Genetics and Genomics, Northern Arizona University, Flagstaff, AZ, USA
- ✓ 30. Antimicrobial susceptibility in goat isolates of *Burkholderia pseudomallei* and molecular database construction of *Burkholderia pseudomallei* isolated from goat using phylogenetic analysis and fingerprinting markers. Yi-Ping Lu et al, Pingtung University of Science and Technology, Taiwan
31. Antigen-capture immunoassay for the diagnosis of melioidosis. D. P. AuCoin et al, . University of Nevada, School of Medicine, Reno, NV, USA
32. Role of the active efflux in doxycycline selected strains of *Burkholderia thailandensis*. Fabrice V. Biot et al, Institut de Recherche Biomédicale des Armées, La Tronche, France
- ✓ 33. Characterization of *Burkholderia pseudomallei* isolates from goat in Taiwan. Ming-Jeng Pan et al, Central Taiwan University of Science and Technology, Taiwan
34. A comparative-genomics algorithm to identify antigenic variability in the core proteome of *Burkholderia pseudomallei*. Daniel Yero, Universitat Autònoma de Barcelona, Spain
35. Assessing *B. pseudomallei* full and exclusive coreproteomes by comparison with *B. Thailandensis*. Oscar Conchillor-Solé et al., Universitat Autònoma de Barcelona, Spain



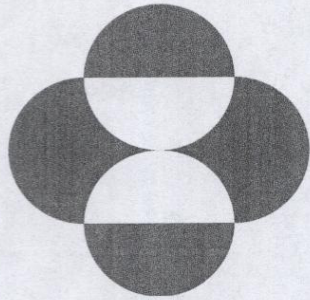
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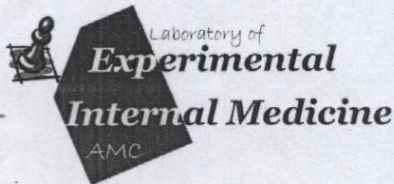
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Poster nr. 30, European Melioidosis Network Meeting

ANTIMICROBIAL SUSCEPTIBILITY IN GOAT ISOLATES OF *BURKHOLDERIA PSEUDOMALLEI* AND MOLECULAR DATABASE CONSTRUCTION OF *BURKHOLDERIA PSEUDOMALLEI* ISOLATED FROM GOAT USING PHYLOGENETIC ANALYSIS AND FINGERPRINTING MARKERS

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Introduction

In 2006, the first infectious case of animal melioidosis was found in a national goat farm located on the southern Taiwan. The antibodies prevalence ranged widely from 26% to 50% during 2006 to 2010. About 54% to 67% deaths of the goats were cultural confirmed as melioidosis. There is no approved vaccine or effective prophylaxis. The delay in diagnosis and lack of knowledge and experience in therapy result in sever outcome of the disease.

Results

The estimated susceptible rate was 80% with Doxycycline and Minocycline, 100% with Trimethoprim/ Sulfamethoxazole by MIC method. When compared the susceptibility by MIC method between filamentary and non-filamentary isolates, we found that filamentary isolates were more susceptible to Cefotaxime, Chloramphenicol and Piperacillin/Tazobactam than non-filamentary isolates, but less susceptible to Amikacin, Gentamicin, Neomycin, Acid, Ticarcillin/Clavulanic Acid, Tobramycin, Amoxicillin/Clavulanic, Tetracycline and Ceftazidime. Based on nucleotide diversity of capsular polysaccharides synthesis associated genes, 15 morphological phenotypes of *B. pseudomallei* were divided into two monophylies, named clade I and clade II. With clade II, it could be subdivided into three subclades, including clade IIA, IIB and IIC. Meanwhile, flagella *fljC* gene and 5 outer membrane proteins genes were divide into 2 clades without subclades causing by low resolution.

Methodology

By using disc diffusion and micro plate MIC methods (Sensititre[®], TRED, England), we perform 53 antimicrobial susceptibility test on the 30 *B. pseudomallei* strains isolated from goats. Susceptibility to antimicrobial agents was evaluated according to CLSI guidelines M31-A3, M45-A2 and M100-S21. We used 15 different morphological phenotypes of *B. pseudomallei* stains isolated from goat for molecular database construction. Based on DNA amplification and sequencing, nucleotide diversity and phylogenetic analyses of 24 capsular polysaccharides synthesis associated genes, flagella *fljC* gene, 7 MLST genes and 5 outer membrane proteins genes were estimated.

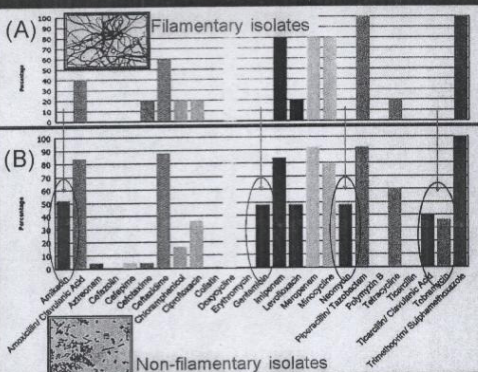


Figure 1. Percentage of susceptibility between filamentary and non-filamentary *B. pseudomallei* strains. (A) filamentary (B) non-filamentary *B. pseudomallei* strains

Table 1. Results of important antimicrobial susceptibility and MIC values on the 30 *B. pseudomallei* strains isolated from goats

	Test No.	S	I	R	NI ^a	≥32	≥32
Amoxicillin / clavulanic acid	30	0 (0.0%)	0 (0.0%)	0 (0.0%)	30 (100.0%)	≥32	≥32
Amoxicillin	30	23 (76.7%)	5 (16.7%)	2 (6.7%)	0 (0.0%)	8	16
Ceftazidime	30	25 (83.3%)	5 (16.7%)	0 (0.0%)	0 (0.0%)	8	16
Ceftiofur	30	0 (0.0%)	0 (0.0%)	0 (0.0%)	30 (100.0%)	≥16	≥16
Chloramphenicol	30	5 (16.7%)	11 (36.7%)	14 (46.7%)	0 (0.0%)	16	≥32
Ciprofloxacin	30	10 (33.3%)	7 (23.3%)	13 (43.3%)	0 (0.0%)	2	≥4
Doxycycline	30	24 (80.0%)	5 (16.7%)	1 (3.3%)	0 (0.0%)	2	8
Imipenem	30	25 (83.3%)	3 (10.0%)	2 (6.7%)	0 (0.0%)	2	8
Levofloxacin	30	13 (43.3%)	11 (36.7%)	6 (20.0%)	0 (0.0%)	4	8
Meropenem	30	27 (90.0%)	2 (6.7%)	1 (3.3%)	0 (0.0%)	2	4
Minocycline	30	24 (80.0%)	2 (6.7%)	4 (13.3%)	0 (0.0%)	2	16
Neomycin	30	12 (40.0%)	1 (3.3%)	17 (56.7%)	0 (0.0%)	16	≥64
Piperacillin / Tazobactam	30	28 (93.3%)	2 (6.7%)	0 (0.0%)	0 (0.0%)	8	16
Tetracycline	30	16 (53.3%)	4 (13.3%)	10 (33.3%)	0 (0.0%)	4	≥16
Ticarcillin / Clavulanic Acid	30	10 (33.3%)	18 (60.0%)	2 (6.7%)	0 (0.0%)	32	64
Tigecycline	30	0 (0.0%)	0 (0.0%)	0 (0.0%)	30 (100.0%)	1	8
Trimethoprim / Sulphamethoxazole	30	30 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.5	1

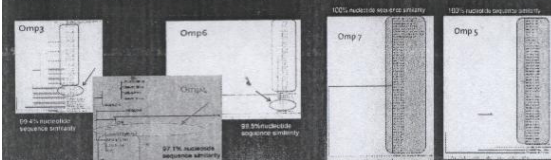


Figure 2. Phylogenetic analysis of goat *B. pseudomallei* isolates based on Omp genes sequences.

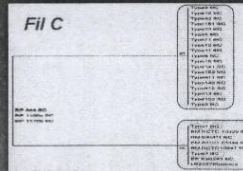


Figure 3. Phylogenetic analysis of goat *B. pseudomallei* isolates based on flagellin protein *fljC* genes sequences.

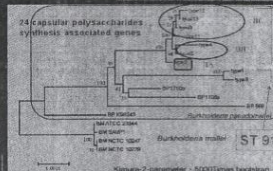


Figure 4. Phylogenetic analysis of goat *B. pseudomallei* isolates based on 24 capsular polysaccharides synthesis associated genes sequences.

Discussion

Disc method of *B. pseudomallei* may be useful as a limited screening tool in resources poor settings because there was no formal interpretation standard for evaluating the test. As a result, some antimicrobial susceptibility may be over- or under-estimated. According to our data, *B. pseudomallei* isolates had good susceptibility to Trimethoprim/Sulfamethoxazole, Piperacillin/Tazobactam, Meropenem, Imipenem, Ceftazidime, doxycycline and minocycline. But Trimethoprim/Sulfamethoxazole and Doxycycline maybe the proper choice of therapy for goat melioidosis. Our results can be further applied for molecular identification based on molecular fingerprinting markers. In addition, our data could be constructing the molecular database for detection of animal *Burkholderia pseudomallei* and studying on relative epidemiology, diagnostic reagents and vaccine development.

Poster nr. 33, European Melioidosis Network Meeting

CHARACTERIZATION OF *BURKHOLDERIA PSEUDOMALLEI* ISOLATES FROM GOAT IN TAIWAN

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Introduction

Burkholderia pseudomallei is the etiologic agent of melioidosis, a severe and fatal disease both in human and animal. In the rainy season of 2006, the first infectious case of animal melioidosis was exploding in a national goat farm located on the southern Taiwan. The infectious disease was recurrence in the goat farm for 5 years and caused economic losses.

Methodology

We isolated the etiological bacteria or detected the genome by real-time PCR from water, soil, home making hay, and various secretions from infected animals (nasal discharge, urine, milk and feces). The isolates from infected goats showed different morphological phenotypes. We screened and collected 300 isolates of goat *Burkholderia pseudomallei*. After purification, we identified and characterized them with API20NE, 16S ribotyping and multilocus sequence typing (MLST).

Results

In our results, over 80% goat isolates were mixed with at least 2 different morphological phenotypes of *Burkholderia pseudomallei*. In our evidences, 15 different morphological phenotypes of *B. pseudomallei* in our isolates from Taiwan cultivated goats. Among them, 2 morphological phenotypes were filamentary. Bacterial identification by API20NE was showing these isolates belong to *B. pseudomallei* (numerical profiles 1556574, 1556575, 1556576 and 1556777). Nucleotide identification based on 16S rRNA gene was showing 100% similarity. In addition, there were identified 4 genotypes (ST57, ST58, ST91 and 1 new ST) by using MLST method.

Discussion

ST91 genotype had reported from American human case, while ST57 and ST58 were widely spread in Asia. ST58 genotype should be more attention because it had been isolated from human melioidosis case in Taiwan. Although these 3 genotypes were formerly reported from human case, there was not any workers infected the disease in this farm in the past 5 years.

Table 1. 15 Different morphological phenotypes of *B. pseudomallei* isolates from goats in Taiwan

Morphological phenotype	Type 1	Type 2	Type 3	Type 4	Type 5
MLST Genotype	ST 91	ST 58	ST 91	ST 58	ST Taiwan New
Colony Morphology on Blood Agar Plate after 10 Days of Growth					
Colony Morphology on Ashdown Agar Plate after 10 Days of Growth					
Morphological phenotype	Type 6	Type 7	Type 8	Type 9	Type 10
MLST Genotype	ST 58	ST 58	ST Taiwan New	ST 57	ST 58
Colony Morphology on Blood Agar Plate after 10 Days of Growth					
Colony Morphology on Ashdown Agar Plate after 10 Days of Growth					
Morphological phenotype	Type 11	Type 12	Type 13	Type 14	Type 15
MLST Genotype	ST 58	ST 57	ST 58	ST Taiwan New	ST 58
Colony Morphology on Blood Agar Plate after 10 Days of Growth					
Colony Morphology on Ashdown Agar Plate after 10 Days of Growth					
Bacterial Morphology under Microscope (Gram Stain)					

Table 2. MLST genotypes reported from human case in Taiwan

MLST Genotype	Number	Percentage	Reported from
ST50	1	9.1%	China, Malaysia, Thailand
ST58	7	63.6%	Malaysia, Thailand
ST67	1	9.1%	Singapore
ST91	1	9.1%	USA, Philippines, Malaysia, Thailand
ST451	1	9.1%	Taiwan

Table 3. MLST genotypes reported from goat case in Taiwan

MLST Genotype	Number	Percentage	Reported from
ST57	2	13.33%	France, Philippines
ST58	8	53.33%	Malaysia, Thailand
ST91	2	13.33%	USA
ST new	3	20%	Taiwan

