

Minutes of the 7th Annual Meeting of the
International *Burkholderia cepacia* Working Group
April 5-7 2002. San Antonio, Texas
Prepared by Paul Whitby

Friday April 5, 2002

Members of the International *Burkholderia cepacia* working group met Friday April 5th in the St Anthony room for informal conversation and dinner. A welcoming address was delivered by Dr. Carlos Gonzales.

Saturday April 6, 2002.

Session I Presentations: The following speakers gave short talks, as detailed below.

John LiPuma Update: US Referral Lab.

David Speert/ Deb Henry Update: Canadian Referral Lab

Susanna McColley Update: Microbiology Laboratory Compliance Review & Reaction to CFF Infection Control Consensus Conference Guidelines

Richard Laing Transient sputum culture of *Burkholderia cepacia* in cystic fibrosis patients: epidemiology and clinical implications

Luigi Chiarini Environmental and clinical isolates of *Burkholderia cepacia* complex: do differences exist between them?

Dr. Lipuma asked if the environmental isolates were clonal or different. Dr LiPuma's experience in the New York state environmental samples were not as diverse as may be expected for environmental isolates. Dr Chiarini did not know about the clonality of their isolates. Dr Mahenthalingam asked where the genomovar IIIa isolates were from since they had not previously been isolated. Dr LiPuma responded that this may be due to certain isolates occupying individual and distinct niches, and that defining the preferred habitat for the BcC would be worthwhile. Dr Parke raised the issue of maceration, previously she had not seen maceration with genomovar III, yet this study demonstrated this. Dr. Gonzalez suggested that this may be due to a methodological detail and was highly dependant on the cultivar of the onion used.

Kathleen Thickett Towards establishing a multilocus sequence typing (MLST) scheme for *Burkholderia cepacia* complex.

Dr. Conway enquired whether there was evidence of gene duplication or were two genes present. Dr Thickett did not know if this was true. Dr LiPuma asked if multiple loci from different isolates had been sequenced to establish diversity of the targets. Dr Thickett replied that this had been done and for the loci studied the diversity was similar to that for recA. Dr. Coenye commented that this approach is expensive per isolate, but yields a very defined result.

Tom Coenye Multilocus restriction typing, a novel tool for studying global epidemiology of *Burkholderia cepacia* complex infection in cystic fibrosis

Philip Sayre *Burkholderia cepacia* -- 2002 Regulatory updates.

Adam Baldwin Characterisation of the *Burkholderia cepacia* epidemic strain marker (BCESM) for virulence factors contributing to the spread of the bacterium among patients with cystic

fibrosis.

Lixia Liu Identification and characterization of a novel insertion element of *Burkholderia cepacia* genomovar III

Emma Rees Biocide and antibiotic susceptibility of chlorhexidine and triclosan-sensitive mutants of *Burkholderia cepacia* K56-2

Barb Conway Is there a role for the *Burkholderia cepacia* mucoid phenotype in CF lung infection?

Roberto Rizzo Exopolysaccharides produced by CF strains of *Burkholderia cepacia*

Joanna Goldberg Lack of correlation of serotype and genomovar in *Burkholderia cepacia* complex

Jacqueline Chung *Burkholderia cepacia* genomovar III persistence in the mouse in relation to colony morphology

Ute Schwab Growth of *Burkholderia cepacia* in airway surface liquid

Chris Mohr Identification and characterization of locus required for cable pilus biogenesis in *Burkholderia cepacia*

Uma Sajjan Identification and analysis of the Cable pili gene cluster in *Burkholderia cepacia*

Melissa Ashlock Status of *Pseudomonas aeruginosa* microarray chips & prospects for *B. cepacia* complex microarray

Jim Tiedje Development of genomic oligonucleotide microarrays for *Burkholderia* sp. LB400

K. Konstantinidis Genomic characterization of *Burkholderia* sp. strain LB400 and comparative analysis with other *Burkholderia* genomes

Following a short break for coffee the delegates split into three moderated discussion groups as detailed. Group A Natural History, Outcomes, Management, Infection Control was Moderated by George Mallory, Group B Clinical Microbiology, Molecular Epidemiology, Taxonomy, Ecology, and Genetics was moderated by Jim Tiedje and David Speert moderated Group C, Virulence Factors, Pathogenesis, and Animal Models.

Sunday April 7, 2002

The presentations are detailed below.

A. Chakrabarty Redox proteins as virulence factors of *Burkholderia cepacia*

Cindi Corbett Cloning of a *Burkholderia cepacia* metalloprotease gene

E. Medrano Genetic characterization of plant tissue watersoaking in *Burkholderia cepacia*

Tom Lessie Analysis of mutant derivatives of *Burkholderia multivorans* and *Burkholderia ambifaria* altered with respect to formation of N-acyl homoserine lactones.

Miguel Valvano New molecular tools for the gene transfer and expression,

and rapid detection of essential genes in *Burkholderia cepacia* genomovars

Paul Whitby Subtractive hybridization of *B. cepacia* genomovar III

The leaders of the breakout groups presented a summary of the discussion.

George Mallory discussed Clinical aspects of natural history, management and outcome and infection control. It was stated that the Toronto ET12 experience is a good source of natural history. Several aspects of infection have been charted. Following acquisition approximately 10% undergo a rapid demise, or “*cepacia* syndrome”, 10% show “no clinical effect”, 60% show a slow deterioration, 10% show slow deterioration followed by rapid demise and 10% show clearance. Questions remaining to be answered included the impact of age of acquisition and FEV₁, the influence of co-infecting organisms (both bacterial and viral) and the influence of host response. Determination of the natural history of other strains and genomovars will require systematic use of reference laboratories by CF caregivers and clinical research with the national databases or multicenter cooperation. Dr. Mallory also stated that the group felt a requirement for the further elucidation of natural history of CF lung transplant recipients with BcC.

With regard to management and outcome issues the group felt there should be investigations into the impact of prescription practice on clinical outcome, such as the change in microbiology, change in antimicrobial susceptibility and morbidity and mortality. The group felt that interesting observations would arise from the MCBT study of Shawn Aaron in Ottawa.

Dr. Mallory discussed linking the clinical and microbial data. For this to happen there were requirements for the development of effective, safe, legal and ethical methodology for permitting the linking of individual clinical data in national databases with BcC identification in repositories. In addition new informed consent protocols need to be developed to permit prospective and retrospective clinical research.

Issues pertaining to infection control were discussed. These included review of isolation practices pioneered in CF centers during the epidemic BcC outbreaks in the 1980s. The proposed extension of isolation practices to include individuals with *Pseudomonas aeruginosa* is presenting US CF centers with a much more onerous and expensive responsibilities. In addition the group felt that there should be prospective research on transmissibility and efficacy of infection control policies as well as a comparison of infection control strategies around the world. Teresa Urban asked whether “*cepacia* syndrome” is seen with all genomovars, Dr. LiPuma replied that while he did not have such evidence, he did have isolates of all genomovars forwarded to his repositories that were originally described as isolated from blood, indicating a serious disease state. David Speert asked what was the current situation in CF institutions regarding segregation? Were people being segregated or being placed into cohorts. It was suggested that someone maybe looking into this as part of a study.

The topics discussed by the Clinical Microbiology, Molecular Epidemiology, Taxonomy, Ecology, Genetics breakout group was presented by Jim Tiedje. With regard to the genome-sequencing project it was suggested that BcC genomovar IIIc environmental strain be added to the proposed list, with *B. gladioli* in reserve, pending whether this organism is sequenced by a Korean group. Dr. Tiedje detailed the group’s discussion on available typing systems. It was concluded that a larger and broader strain panel is required to cover the entire *Burkholderia* group and that this should contain environmental isolates. It was also stated that specific PCR primers for genomovars V and III a, b, and c should be developed. The dot blot membranes currently being developed by Dr. Mahenthalingam were discussed as a useful tool both for epidemiology and typing.

Dr Tiedje presented the groups recommendations on molecular epidemiology. It was felt that more information is required to better address the relationship between human and environmental sources of BcC and that other issues such as reservoirs, infectious dose, antibiotic resistance patterns and markers for recent infection need to be determined.

The main points of the breakout groups discussion on genetics were that a better understanding of the degree of horizontal transfer of virulence factors and other traits was required, and that this needed to be extended to elucidate other mechanism of transfer, such as phages. The group felt that information might be derived from comparative genomics to provide ideas that may be used to formulate and pursue specific hypotheses.

Dr Tiedje discussed several proposed post-genome activities. These included the annotation of the available data with the establishment of a centralized body for the regular maintenance of a current gene database, similar to that established for *P. aeruginosa*. The group also felt there was a requirement for arrays to be developed to allow pursuit of various goals across several organisms.

Finally the group felt that the strain panel required both revision and expansion to include more environmental isolates and the new genomovars.

Dr. Speert presented the conclusions of the Virulence group. The main thrust of the groups discussion was establishing definitions for pathogenesis and virulence in the context of BcC infection. Dr. Speert detailed the thoughts of the group. With regard to available models, there were parallels between animal and plant responses to “virulent” isolates of BcC. In onions the more virulent isolates lead to a hypersensitivity response, limiting pathology. In the BALB/c mouse model, utilized by Dr. Speert, genomovar III was more rapidly cleared than a *B. multivorans* isolate. Thus, the group concluded appropriate alternative endpoints need to be determined for individual models. Such endpoints could include persistence, cellular response, humoral response and/or invasiveness. The group’s discussion in this area was summarized by Dr. Schwab who stated “ We really don’t understand what happens in vivo, so it is difficult to develop a logical animal model”. The group also concluded that focus should shift to examine host determinants of BcC infection. Dr. Speert detailed the group’s recommendations for future research. These were to develop relevant chronic infection/colonization models, evaluate host determinants that enhance susceptibility to BcC, develop microarrays to study animal infection and begin establishing proteomics based studies.

Final discussion of the group included a short talk by Paul Whitby, who informed the group that the IBCWG webservice had been established. He asked the group if they would all like to be added to the servers mailing list and received an affirmation from all present delegates.

The last item to be discussed was the site and dates of the next IBCWG meeting. Eshwar Mahenthiralingam, Mary Corey and Paul Whitby are investigating potential venues and will contract the group once a decision is made.

Meeting adjourns and the delegates meet for lunch in the St. Anthony Club prior to departing.