

Minutes of the Eighth International *Burkholderia cepacia* Working Group Meeting
Thistle Hotel, Tower of London, London
March 21-23, 2003

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Program and Abstracts available online at: <http://go.to/cepacia>

Opening Session, Friday 21 March

The meeting sponsors: AstraZeneca Canada and United Kingdom, US Cystic Fibrosis Foundation, Canadian Cystic Fibrosis Foundation, UK Cystic Fibrosis Trust and Cardiff University, Cardiff, Wales, UK, were acknowledged.

Referral laboratory report highlights:

- **US Referral Laboratory** (Tom Coenye on behalf of John LiPuma). Current statistics of infected patients: 44% *B. cepacia* genomovar III, 36% *B. multivorans*, 5% *B. cepacia* genomovar I, 5% *B. vietnamiensis*, 10% remaining genomovars. Also several major clones have now been found to infect multiple patients such as the PHDC clone (*B. cepacia* genomovar III-B, cable pilus negative, BCESM negative) which infects greater than 200 patients in 24 US states. Other instances of shared strains between patients have been found for *B. cepacia* genomovar I, *B. multivorans* and *B. cepacia* genomovar VI.
- **Canadian Referral Laboratory** (David Speert). An instance of patient-to-patient spread of *B. multivorans* occurring among Canadian patients was described for the first time.
- **UK Referral Laboratories: Scotland** (John Govan) encountering an average incidence of approximately 4% of patients infected with BCC with some centres as high as 8%. **England and Wales** (Fiona Clode) - approximately 157 isolates obtained from 41 UK hospitals; *B. multivorans* form the majority of isolates encountered (45%) with *B. cepacia* genomovar III accounting for 21%. Notably, *B. gladioli* (12%) and *Pandoraea* (8%) were also prevalent isolate types in this collection.

Taxonomy highlights (Peter Vandamme):

- Nine genomovars now constitute the *B. cepacia* complex
- *B. cepacia* genomovar III has been formally named as *B. cenocepacia* spp. nov.
- A proposal to name *B. cepacia* genomovar VI as *B. dolosa* is currently in preparation.
- *Burkholderia ubonensis* looks like it will constitute *B. cepacia* complex genomovar X

- A *B. cenocepacia recA* subgroup III-A isolate has been recovered from river water indicating that this strain lineage may be found in the natural environment.

Presentation of abstracts:

The abstracts shown in the final program were presented except for abstracts: A27, A34, A18, A41, A11, and A30. Luigi Chiarini kindly presented abstract A7 at short notice. Abstract A25 was presented by Peter Vandamme on behalf of Silvia Campana and abstract A33 was presented by Uma Sajjan on behalf of Teresa Urban.

Summary of discussion sessions:

Discussion sessions were not held in separate small groups this year. Instead all participants discussed each topic area in turn. Summaries of each were as follows:

1. Clinical Issues (Chaired by Andrew Jones)

Cross infection control issues

- **Criteria for patient status as *B. cepacia* culture negative.** If a patient is culture positive for *B. cepacia* then appropriate decisions for the clinical management of such a patient may be made. However, patients previously culture positive for *B. cepacia* present a management dilemma in terms of infection control and therapy practice. Recent prevalence data indicate that the numbers of CF patients falling into this category of "infection status" may be increasing. Early aggressive antibiotic therapy appears to lead to eradication of infection in certain cases of *B. cepacia* complex infection.
- Most guidelines recommend that a patient only be considered culture negative if three sputum cultures taken over a period of at least 12 months fail to grow *B. cepacia* bacteria.
- It was noted that some patients become culture positive after over a year of being culture negative.
- Routine screening varies from clinic to clinic, but, screening previously *B. cepacia* positive patients at least once every three months seemed an appropriate minimum recommendation. Co-operation with patients would be needed if this was to be made a formal recommendation.
- It was also not known if re-appearance of *B. cepacia* in such patients constituted a re-growth of the previously infecting strain or acquisition of a new infection. Additional unknowns raised were: (i) Are genomovar differences apparent in reacquisition? (ii) Is infection really lost or has *B. cepacia* become dormant?
- PCR based screening may be advantageous for culture negative patients. However, the group noted that sensitivity of such PCRs were questionable. Whether a PCR positive but culture negative patient posed an infection control risk could never be systematically tested.
- These discussions raised an area of *B. cepacia* epidemiology which requires urgent systematic testing to enable better clinical management of CF patients. It

was noted that several PCR tests were available to answer such questions in a prospective study of CF patients infected with *B. cepacia*.

***B. cepacia* complex and other species diversity – clinical risks?**

- Co-infection of *B. cepacia* and other *B. cepacia*-like species is sometimes seen. How many cohabiting species are seen? How often do the species change during infection? What are the clinical risks from some of these other species? Do we need to keep screening *B. cepacia* complex positive patients to see if they acquire a different strain or genomovar that would not be detected by routine phenotypic identification? If this is done, how often should it occur? There is certainly a need for continued surveillance, but this may not be available to many clinics
- Overall, the group had no immediate answers to these questions and once again indicated that these issues were important areas for further study.

Transplantation

- Several studies have now demonstrated that transplantation of *B. cepacia* positive patients is associated with poor outcome, especially, though not absolutely, if the infecting strain was *B. cepacia* genomovar III. What should be the recommendation for transplantation of infected patients?
- Several centres simply do not transplant *B. cepacia* positive patients
- Other centres such as Toronto continue to transplant patients irrespective of *B. cepacia* infection status
- It was recommended that patients should be informed of the increased risks associated with *B. cepacia* infection and then given the responsibility to decide for themselves whether to proceed with transplantation
- *B. cepacia* positive patients should be offered transplants and prioritised in same way as all patients being offered this therapy.

Early Infections and Eradication

- Aggressive antibiotic therapy appears capable of eradication of early *B. cepacia* infection in several centres.
- This requires the administration of a minimum of three antibiotics to which the infecting strain is susceptible, although the optimal regime is not known
- It was not known if this regime is effective against all *B. cepacia* complex genomovars or whether nebulized antibiotics are effective at eradication of infection.
- This discussion highlighted another area of *B. cepacia* infection which requires further study or trials to identify best practice.

Burkholderia gladioli

- This organism appears to be problematic in several UK centres

- Severe infection is associated with poor clinical course and the formation of multiple extrapulmonary abscesses has been reported.
- These problems raise the question of how both patients and clinicians view *B. gladioli* infection. Greater risk is always attributed to *B. cepacia* infection.
- *B. gladioli* infection does not appear to present a significant infection control risk.
- However despite being susceptible to more antibiotics than most *B. complex* bacteria, infection may be very difficult to treat.
- It was noted that many centres may have poor resources to identify *B. gladioli*.

‘*B. cepacia* Syndrome’

- How can this be treated?
- The group could not offer any further suggestions.
- Immunomodulatory therapy has been tried.
- Antibiotics have limited efficacy at this stage.
- It was noted that administration of Manuka honey may have been tried.
- It was asked at what stage of *B. cepacia* syndrome would clinicians try an experimental therapy? Potentially, a very early stage was the answer. A more systematic approach to documenting and reporting treatment of "*B. cepacia syndrome*" was recommended and that this information could be collated among the IBCWG.
- Questions deserving of further study were raised: What is phenotype of bacterium during cepacia syndrome? What is the trigger? Host versus the bacterium versus the environment? Can *B. cepacia* syndrome be predicted? What are the markers?

Issues in Taxonomy, Epidemiology and Ecology (Chaired by Tom Coenye)

Identification – is there a need for “minimal standards”?

- Recommendations?
- Several useful biochemical tests available
- PCAT medium prevents growth of *B. cepacia* genomovar VI (*B. dolosa*)
- Molecular assays still required for many other genomovars.
- Clinical microbiology labs still pass on burden (and cost) of identification to a referral laboratory.

Epidemiology

- Several genetic typing methods have now been shown to be useful for epidemiological analysis of *B. cepacia* complex infection. These include pulsed-field gel electrophoresis, Repetitive Element PCR fingerprinting, Random Amplified Polymorphic DNA (RAPD) fingerprinting, Amplified Fragment

Length Polymorphism analysis. All have been applied successfully to the *B. cepacia* complex.

- A unified MLST approach being developed by Chris Dowson's group will offer potentially greater versatility in the future analysis of data and determination of the evolutionary relationships between genomovars. Several IBCWG participants were interested in this initiative and it was hoped the scheme could be formally funded and developed in the next year.

Ecology & Identification

- Isolation of all *B. cepacia* complex bacteria from the natural environment still requires further exploration ie. what protocols are appropriate for what environments?
- Is there a variation in culture methods required for certain genomovars?
- Dormancy and relationship to environmental temperature. It was noted that below 18 to 20 degrees *B. cepacia* bacteria are very difficult to culture.
- It was noted there could be more use of non-culture DNA based approaches. The group were all in favour of this approach, however, noted that isolates were always needed. Considerable information could be gained from non-culture based molecular screens especially where culture based approaches have failed to isolate organisms to date.
- What non-culture tests could be applied? 16S rRNA PCRs and *recA* PCRs could both prove very useful.
- It was noted that the genomovar specific *recA* primers did not show 100% specificity for certain genomovars such as III-C and III-D subgroups of genomovar III. In addition, it also appears that *B. cepacia* genomovar I is very diverse in terms of *recA* polymorphisms and the current specific primers for this group were no longer satisfactory.
- If measures of diversity were needed, MLST could provide a future means to examine environmental distribution of the *B. cepacia* complex.
- Reservations were expressed about different culture isolation methods and that these could still be further enhanced to enable capture of bacteria from the natural environment.
- It was concluded however, that certain groups will always use certain isolation methods and that this could not be standardised through IBCWG recommendations. The post-isolation identification procedures would be easier to standardise especially in light of the minimum recommendations stated above.

Genomics, Molecular Biology, Infection

(Chaired by E. Mahenthiralingam and including a summary of discussions from the *Burkholderia* Genomes meeting held at the Sanger Institute, March 24-25, 2003)

Genomics

- New genome sequencing projects. As a result of discussion initiated during the San Antonio IBCWG meeting and the efforts of Prof. Jim Tiedje in co-ordinating

- a sequencing proposal, the US Department of Energy Joint Genome Institute has agreed to sequence at least three further *B. cepacia* complex bacteria.
- Two initial strain choices have been made: (i) *B. vietnamiensis* strain G4 (ATCC 53617), a well studied bioremediation strain and (ii) *B. ambifaria* ATCC 17760, a strain with the smallest *B. cepacia* complex genome characterised to date.
 - Tom Coenye has prepared the DNA from each isolate culture deposited in the BCCM LMG collection. The DNA is now at the Joint Genome Institute and sequence analysis should start shortly. All draft data will be made publicly available as for the *Burkholderia* species strain LB400 genome project

Comparative genomics of *B. cepacia* genomovar III strains related to J2315

- Several genomovar III strains are used by different investigators as laboratory models - how closely related are they in terms of sequence and ability to manipulate in the lab?
- Model strains used include: K56-2 and BC7 (both ET12 lineage), and H111 (genomovar III-A, but exact strain status is not known)
- To date, most investigators noted that all structural genes sequenced for these strains were identical to the genome sequence.
- Minor variations in PFGE profile are apparent in the two ET12 strains K56-2 BC7 suggesting that some loci will show rearrangements, but these have not been observed at the sequence level as yet.
- Chris Dowson noted that from MLST data he would expect less than 1-2% nucleotide sequence divergence in 400-500 bp fragments of housekeeping genes within a single genomovar; this rises to 4-8% between genomovars.
- The high levels of sequence identity with J2315 suggest it was a good choice for sequencing.
- The ability to genetically manipulate the "isolate" of J2315 possessed by Chris Mohr's group is also being further investigated. By PFGE this "isolate" shows a one band difference with the J2315 isolate that was sequenced.

Annotation of J2315

- Several groups have begun annotation of regions of J2315 genome that interest them
- At the genome meeting, the complete annotation of *B. pseudomallei* was presented
- Julian Parkhill (Microbial Pathogen Sequencing Unit, Sanger Institute) noted that the annotation of *B. cepacia* strain J2315 will now commence, transferring as much of the *B. pseudomallei* annotation over as possible.
- Discussions among the group noted that researchers should wait for the Sanger Institute annotation as it will be more thorough and systematic than any automated computational annotation. It will also produce a standard gene numbering system and nomenclature for the J2315 genome. Most investigators already find the taxonomy and strain naming for these bacteria difficult and complex. It would make a lot of sense to just work from one annotation for all

- genomics related projects such as arrays (see below). The full annotation should be available by Autumn/Winter 2003 when the genome sequence is published.
- **Maintenance and updating of the annotation?** At the genome meeting Julian Parkhill indicated that the Sanger Institute were working on database models to allow updating of their genome annotations using a software platform that was being developed (<http://www.genedb.org>). This appeared to be the best way forward for the IBCWG/*B. cepacia* community in terms of maintaining the J2315 annotation. Discussions among the group noted that this may be an area to solicit international funding for a dedicated post to update the *B. cepacia* J2315 annotation in conjunction with the Sanger Institute program.

Genome Chips/Arrays, transcriptomics, and comparative genomics

- Melissa Ashlock (US Cystic Fibrosis Foundation) conveyed (via E. Mahenthiralingam) that the Foundation will fund a program to produce a *B. cepacia* J2315 genomic array for the CF research community.
- Two platforms were being considered: (i) An Affymetrix GeneChip analagous to that for *P. aeruginosa* strain PAO1 (**pros:** tried, tested reproducible; **cons:** very expensive development and usage costs) and (ii) An Agilent Technology glass slide based array which uses inkjet technology to synthesise oligonucleotide microarrays directly onto the glass (**pros:** much cheaper development and purchase cost, greater flexibility in producing updated arrays, more user friendly in terms of requiring less specialise/expensive equipment to hybridise and read arrays; **cons:** relatively untested, though early yeast data is impressive).
- **Glass-slide arrays offer the most useful format for the *B. cepacia* community.** Discussions at both IBCWG and the Wellcome Genome Meeting concluded that the glass slide based arrays offer considerable cost saving and more overall flexibility to the research community. Most institutions now have hybridisation stations and readers that can handle such arrays, while the dedicated Affymetrix systems are beyond the reach of many labs. Tying in with Affymetrix was thought to be restrictive and too expensive for most researchers. Overall, the Agilent platform, although untested, was considered to be the most desirable of those currently under consideration.
- **Sanger Institute annotation to form the basis for the array.** The group discussion concluded that the Sanger Institute annotation should form the basis for the array design. Julian Parkhill indicated that they were willing to work with interested parties prior to formal publication of the annotated genome to aid array design.
- **What genes should be included on such arrays?** Genes from other genomovars that are within Genbank. All common antibiotic marker genes, reporters such as GFP and lux would be useful controls. **Could the arrays be expanded further?** For example Jim Tiedje's group are working on arrays for strain LB400 - could we produce combined arrays? Again a major advantage of the glass slide platform is the fact that array design may be updated/expanded very easily.

Vectors and Tools:

- Miguel Valvano's group were working on vectors to enable rapid low-copy number cloning and complementation of genes *B. cepacia*.
- **Unmarked gene knockouts.** E. Mahenthalingam noted that while systems for making marked site-directed gene knockouts were well developed, making unmarked mutations was relatively unexplored in *B. cepacia*. Pam Sokol noted that she thought Herb Schweizer may be developing such systems for *Pseudomonas*.

Infection models:

- Uma Sajjan's model of chronic colonisation in CFTR mice showed considerable promise.
- Miguel Valvano noted that the natural environment should also be considered a model of *B. cepacia* "virulence" or survival.
- Leo Eberl's work has shown that the nematode may be a useful *B. cepacia* model

IBCWG Organisation Issues

1. Future Meetings:

- **Dates - as before.** The end of March or the beginning of April were still considered to be the best time to hold the annual meetings of the group. Also the weekend format works well for international travellers
- **Duration - possibly one additional day?** Given the interest in the field and possibilities of increased participation it was proposed that future meetings commence on Friday and end midday on Sunday.
- **Venues and timeline - outlined to 2007.** The following schedule was outlined to enable the group to work towards each meeting in a co-ordinated fashion:

2004 Vancouver, BC, Canada. To be organised by David Speert.

2005 Oklahoma City, Oklahoma, USA. To be organised by Paul Whitby

2006 Europe. Possibly Prague, Czech Republic. This meeting will be dependent on obtaining sufficient sponsorship and grants from international agencies such as NATO.

2007 Ann Arbor, Michigan USA. To be organised by John LiPuma.

- **Participation - perhaps no size limit? (other than venue size and that set by the local organisers).** After much discussion it was decided that the group cannot limit itself in light of the increasing interest in the field. Hence for future meetings there would be no size limit other than that of the venue.

- **Funding of participants - to be entirely self-funded.** Previous meetings have always subsidised the accommodation costs of invited participants. It was decided to move away from this model to reduce costs and the responsibilities of the organiser. Transferring the cost and responsibility to book accommodation to the participants will significantly reduce the organisation required and work to limit the size of meeting.
- **Expression of Interest Form on website.** Groups interested in participating in future IBCWG meetings could register their interest by completing an online "Expression of Interest Form" on the IBCWG website. This form would have to be very carefully designed such that only truly scientific enquiries could be submitted. This form should be completed by both old and new participants to register their interest and facilitate organisation.
- **Deadlines and Instructions.** A clear series of instructions and deadlines for registration and booking of future meetings should be placed on the IBCWG website prior to each meeting.
- **Meeting scholarships?** If funds were available (perhaps that saved by not subsidising invited participants), then travel scholarships could be offered to junior researchers selected from the abstract submissions.
- **Meeting discussion groups.** This year the discussion sessions were held as one group and not split into subgroups. The framework of points provided by each session chair enabled excellent discussions to proceed and we suggest this as an excellent model to use for future meetings.
- **No formal IBCWG meeting at the Annual North American CF Conferences (NACF).** The group agreed that the formal short meeting of the IBCWG at NACF should cease as they are often very difficult to attend, organise and severely limited by time. An informal gathering of the organising group at a hotel/bar/restaurant may be the best way to interact at future NACF meetings and plan ahead.

Acknowledgements

We thank Tom Coenye for once again doing an excellent job in maintaining the IBCWG website and dealing with the abstract submission. The help from all the additional organisers in drafting the meeting format and running sessions was excellent. Adam Baldwin did a great job in noting down the points from the discussion groups and providing the template for these minutes. Finally, we thank Peter Baldwin (CharterTravel Ltd.) for his professional organisation skills that kept the whole venue and meeting on track.